Natural history of Malagasy poison frogs: experimental analysis of aposematism, morphology of tadpoles, and longevity

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“Theories pass. The frog remains.”

Jean Rostand (1894-1977)

Content

1 Introduction

Natural selection is the most powerful force shaping the evolution of life on Earth. It is not always predictable, and the explanation of various traits evades any definitive answer. The scarcity of samples available does not help the study because all the preceding life forms left no or very meager cues.

One example of such trait is aposematism - described for the first time by Alfred Russel Wallace more then a century ago, but is still one of the hot topics in evolutionary biology. Aposematism is an antipredatory defence adaptation in a potential prey organism that associates the presence of a warning signal (most commonly bright colouration) with unprofitability to predators (such as unpalatability or noxiousness; Cott 1940; Edmunds 1974; Guilford 1990). This phenomenon is based on the psychology of learning and reinforcement of predators, and it has been experimentally well supported that predators learn more readily to recognize unpalatable prey when being conspicuous (e.g. Sillén-Tullberg 1985; Guilford 1990; Guilford 1992; Terrick *et al.* 1995; Gamberale and Tullberg 1996). On the other hand, fixation of this trait represents an evolutionary paradox; the first individuals that developed conspicuous coloration were exposed to increased initial predation. There are several models that try to explain how aposematism could evolve and become stable; kin selection (Fischer 1930; Leimar *et al.* 1986) and "green beard" effect (Guilford 1988; 1990), gregariousness compared to individual selection (Sillén-Tullberg 1985; 1993; Lindström *et al.* 2001), "peak-shift" (Lindström *et al.* 1999), maternal effect (Brodie and Agrawal 2001), and others. Apart from aposematism, another intriguing evolutionary question is the origin of mimicry. Batesian mimicry (Bates 1862), when palatable mimic resembles a conspicuous noxious model, and Müllerian mimicry (Müller 1878; 1879) when both mimic and model species share the same aposematic pattern and benefit from it, are the most common types of mimic.

Moreover, little is known how predators process visual and chemosensory cues about aposematic prey, and it is unclear how this processing interacts with recognition and avoidance of such prey (Guilford 1992; Terrick *et al.* 1995). In general, conspicuousness of a prey is a reliable indicator of its unpalatability and/or noxiousness (Sherratt 2002). The aposematic colouration can deter the predator, although the degree of noxiousness may not be high.

1.1 Aposematism in frogs

Anuran amphibians are organisms well suited for studies of vertebrate evolutionary patterns and numerous publications explore general evolutionary processes in anuran amphibians (e.g. Bossuyt and Milinkovitch 2001; Cannatella and Hillis 1993; Cannatella and Trueb 2008; Hanken and Wake 1993; Hayes T.B.1997; Stöck *et al.* 2006; Vences *et al.* in press; Wake 1970; Wollenberg *et al.* 2008). Anurans also display a high degree of morphological convergence which extends to their coloration.

The colour of anuran amphibians are generally considered to be cryptic, although some taxa are considered to be aposematic (Santos *et al*. 2003; Vences *et al*. 2003; Darst *et al*. 2006). Apart from pigments and numerous biologically active compounds (e.g. peptides), a few groups of frog contain alkaloids in their skin that they accumulate and sequester from the diet into their skin glands (Daly and Myers 1967; Daly *et al*. 1999; Saporito *et al*. 2007a). There are four lineages of alkaloid containing frogs, all of them also having a largely aposematic colour: the well-known dendrobatid frogs, the bufonid genus *Melanophryniscus* from South America, the mantellid genus *Mantella* from Madagascar, and the myobatrachid genus *Pseudophryne* from Australia (Daly *et al*. 1984; Daly *et al*. 2002; Daly *et al*. 2008). The genus *Mantella* is an impressive example of convergence with the poison-dart frogs (Dendrobatidae), also based on their morphological features and complex mating behaviour (Vences *et al*. 1997; 2004; Heying 2001; 2004). Again, while many data on dendrobatid frogs are already published and

known, only a handful of informations have become available so far on the ecology and behaviour of *Mantella*.

1.2 Mantella

The genus *Mantella* comprises attractive aposematic, small diurnal frogs. *Mantella* belongs to the Malagasy-Comoran endemic family Mantellidae. This well defined monophyletic group includes about 17 species that are morphologically poorly differentiated in their adult stage. Based on molecular phylogenetic analyses the genus is further subdivided into five monophyletic groups: the *M. cowani* group (comprising the species *M. cowani, M. baroni, M. haraldmeieri* and *M. nigricans*), *M. bernhardi* group (*M. bernhardi*), *M. madagascariensis* group (*M. aurantiaca, M. crocea, M. madagascariensis, M. milotympanum, M. pulchra*), *M. laevigata* group (*M. laevigata* and *M. manery*) and *M. betsileo* group (*M. betsileo, M. ebenaui, M. expectata, M. viridis, M.* aff. *viridis*) (Vences *et al.* 1999; Schaefer *et al.* 2002; Chiari *et al.* 2004; Rabemananjara *et al.* 2007). Snout-vent length (SVL) is 18–31 mm. Because of their aposematic coloration, *Mantella* are highly priced in pet trade, particularly the more brilliantly coloured species, such that large numbers of specimens are exported from Madagascar every year (Behra 1993; Rabemananjara *et al.* 2008b). In a concerted effort to monitor the trade, all *Mantella* species have been placed on Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Vences *et al*. 2004; Andreone *et al*. 2006).

My research refers mostly to three species of *Mantella*: *M. aurantiaca*, *M. baroni* and *M. madagascariensis*. *M. aurantiaca* is distributed in the northern central east of Madagascar (Bora *et al*. 2008), *M. baroni* stretches from the northern central east further into the south, including Ranomafana and Andringitra National Parks, while *M. madagacariensis* is found in the northern central east and in Ranomafana (Jovanovic *et al*. 2007). All *Mantella* species have a very patchy distribution, and the latter two species

live in some areas in syntopy. Additionally, these two species probably represent an example of Müllerian mimicry (Schaefer *et al*. 2002).

1.3 Madagascar

Madagascar is famous for its biodiversity and high degree of endemism (Goodman and Benstead 2005) and because of its geological history, with an early separation from the Gondwana supercontinent, it is often described as evolutionary model region for explaining different influences on diversity patterns. It also constitutes a natural laboratory for the study of species diversification mechanisms (Vences *et al.* in press). Currently 244 Malagasy amphibian species are described. However, based on the DNA sequences of 2850 specimens from over 170 localities throughout Madagascar, the expected number of amphibian species on the island is estimated between 373 up to possibly 465 or more (Vieites *et al*. 2009). Equally important, is its endemism - all amphibian species are native to the island (Glaw and Vences 2007; Vieites *et al*. 2009). The most prominent is the family Mantellidae that represents the most species-rich endemic frog radiation of Madagascar and currently contains about 240 species (Glaw and Vences 2007).

In accordance with the general high biodiversity in Madagascar, also Malagasy reptiles are species rich, with 370 described species of which 92% are endemic. Most of the Malagasy snakes, which are relevant as predators of frogs, belong to the family Colubridae with 18 genera and 75 species in Madagascar (Glaw and Vences 2007) which is the biggest snake family worldwide and today is often partitioned into several separate families. All snake species used in my study are colubrids.

Due to alarming rates of habitat destruction in Madagascar, the region has been classified as one of 25 most important hotspots for the conservation of biodiversity, where exceptional concentrations of endemic species are undergoing enormous loss of habitat. As many as 44% of all species of vascular plants and 35% of all species in four vertebrate groups

are concentrated in these hotspots comprising only 1.4% of the land surface of the Earth (Myers *et al.* 2000).

1.4 The aim of my research

The genus *Mantella* can be labelled as model group for studying the evolution of aposematism because of (1) great morphological convergence on one side, and (2) colour polymorphism on the other side.

(1) A remarkable degree of convergence exists in colour pattern among species of *Mantella* that occur syntopically. Over the whole of Madagascar, it is common to observe two or three species of this genus at one site, but these syntopic species almost always belong to genetically well-differentiated species groups and are not closely related among each other. Nevertheless, they often show a very strong similarity of color and pattern, often that much that a reliable identification is only possible by examining the ventral pattern and/or molecular and bioacoustic characters. The most striking example is that of *Mantella madagascariensis* and *M. baroni,* but also the species *M. pulchra* and *M. nigricans*, and *M. laevigata* and *M. manery,* as well as *M. aurantiaca* and *M. milotympanum* although these latter two species have not yet been found in close syntopy. Since all *Mantella* are known to contain alkaloids, these examples should most likely be seen as examples of Müllerian mimicry which otherwise is exceptional in vertebrates.

(2) Aposematic theory predicts that, in one population, the colour and pattern of prey specimens should be very stable and uniform, to increase the learning effect with predators. However, in *Mantella*, some species do not only show a high colour polymorphism between but also within populations, which does not seem to be related to genetic polymorphism (Chiari *et al.* 2004).

In this dissertation I studied several aspects of the biology of *Mantella* frogs. Although not so complex, the morphology of *Mantella* is very important. All *Mantella* species have been at least briefly described

morphologically (Vences *et al.* 1999) but this is not true for their larval stages (tadpoles). The morphology of tadpoles was known only for four *Mantella* species, so in this dissertation I filled the void for seven other species. Additionally, I compared tadpole morphology of all these species in a phylogenetic context (Chapter 1).

Chapter 2 presents the results of *Mantella* longevity and tries to contribute to the understanding of possible correlations between the age and toxicity of individuals. These data were obtained during several months of histological lab work.

Longevity and morphology data provide new perspective for conservation of *Mantella* frogs. Longevity in combination with population size, survival rate etc. can help in establishing the influence of harvesting and the recovery time for affected populations. Tadpole morphology data are useful for identification of species in the field, and can help in monitoring the populations.

After providing general information on *Mantella* (Chapter 1 and 2), I focus in the subsequent parts of my study on the aposematic colouration and predation on *Mantella*. In order to examine the effectiveness of the aposematic colouration in *Mantella*, I performed a field study in Madagascar using clay models (Chapter 3). Despite the great sample size (more then 2000 models in two seasons), the results did not completely fulfil my expectations. Since it was a field experiment, I have to stress that any result is a worthy result, even a negative one.

On the other hand, the results of snake feeding experiment (Chapter 4) almost in total supported my hypothesis. I expected to find a significant difference between snake predation upon *Mantella* and edible non-conspicuous frog, as well as an initial difference between "experienced" (that live in *Mantella* habitat) and naïve snakes (that never encountered *Mantella* in the wild).

During my fieldwork in Madagascar I witnessed a rare predation event on *M. aurantiaca* by a colubrid snake which I report in Chapter 5.

Altogether, this dissertation gives a new insight on the natural history of *Mantella* frogs. On one hand, very general aspect is studied, such as tadpole morphology and longevity; on the other hand, the complexity of aposematism and predation of *Mantella* frogs is examined. Both of these aspects put *Mantella* frogs in broader perspective and provide a basis for further research.

2 Comparative larval morphology in Madagascan frogs of the genus *Mantella* (Amphibia: Mantellidae)

Abstract

I describe and compare the tadpole morphology of nine species of frogs of the endemic Madagascan genus *Mantella* based upon specimens identified through DNA barcoding or captive bred. The tadpole morphology of *M. crocea/milotympanum*-hybrids, *M. madagascariensis*, *M. pulchra*, *M. viridis*, *M. baroni*, *M. bernhardi* and *M. betsileo* is described for the first time. In general, *Mantella* have small and generalized tadpoles with a uniform dark colouration. The oral disc is elliptical, emarginated, and positioned anteroventrally. In *M. laevigata* the oral disc is rounded, not emarginated, and positioned ventrally; eyes are positioned and directed dorsally, while in other species they are directed dorsolaterally. Labial tooth row formulas of *Mantella* tadpoles differ among some species, and in *M. aurantiaca* and *M. crocea/milotympanum* they also show intraspecific variation. Species identification is difficult when considering only morphometric variables. Tadpoles within each species group of the genus do not cluster together (except for some clustering of species belonging to the *M. madagascariensis* group), confirming that the larval morphology in closely related *Mantella* species is not suitable for determining phylogenetic relationships. *Mantella laevigata*, distinguished by tree-hole breeding and parental care, shows the most distinguished morphology.

Keywords: Anura, tadpole description, DNA barcoding, Madagascar, morphometry, ontogenetic variation

2.1 Introduction

The genus *Mantella* comprises attractive, small diurnal frogs which accumulate skin alkaloids, and are characterized by aposematic colouration (Daly *et al.* 1996; Vences *et al.* 1999). *Mantella* are highly priced in the pet trade, particularly the more brilliantly coloured species, such that large numbers of specimens are exported from Madagascar every year (Behra 1993; Rabemananjara *et al.* 2008). Despite of their commercial interest and the fact that many publications are available on the husbandry of most of the species, it is surprising that detailed tadpole descriptions are only available for *M. aurantiaca* by Arnoult (1965), later summarised by Blommers-Schlösser and Blanc (1991), and *M. expectata* (Mercurio and Andreone 2005), while for two other species (*M. ebenaui* and *M. laevigata*) only rough descriptions have been published (Glaw and Vences 1994).

On the other hand, seen the very high number of known species of amphibians in Madagascar, it is not strange that for most of them the tadpole morphology and general larval ecology are not yet known. Notwithstanding, the knowledge of larval stages is a crucial step in the assessment of conservation priorities, and only the analysis of all life-history stages of a species results in a clear picture of the ecological requirements of a species. This is in particular true for anurans because tadpoles are known to be highly adapted in morphology and ecology to local ecological conditions (Mercurio and Andreone 2005; Candioti 2007).

This high level of adaptations to their environment was seen as a main factor causing the morphology of anuran larvae to reflect only poorly their phylogenetic relationships. However, several recent papers have shown that tadpole characters are phylogenetically informative (e.g. Maglia *et al.* 2001; Haas 2003; Grosjean *et al.* 2004). Due to the entirely different organisation of anuran larvae, the characters of tadpoles are complementary to those of adults and this set of new characters could help to resolve taxonomic and phylogenetic problems where adult characters alone have been inadequate (Grosjean 2005). Here I provide descriptions of the tadpole morphology of

nine species of *Mantella*, six of them for the first time. Additionally I compare the external morphological characters and oral disc morphology between different species, as well as morphological measurements, as a contribution to an inventory of Malagasy anuran larval stages.

2.2 Materials and methods

Tadpoles were either collected in the field or reared after captive breeding. All animals were euthanized by immersion in chlorobutanol solution, and animals captured in the wild were immediately sorted into homogeneous series based on morphological characters. F1 hybrid tadpoles between *M. crocea* and *M. milotympanum* were obtained by captive breeding.

Tadpoles collected in the field were identified using the DNA barcoding approach, a rapid molecular technique that has shown reliable results in amphibian species identification (Vences *et al.* 2005a, b). We used a fragment of the mitochondrial 16S rRNA gene that is known to be sufficiently variable among species of amphibians (Vences *et al.* 2005a, b).

All specimens are deposited at the Zoologische Staatsammlung München (ZSM). Developmental stages are based on Gosner (1960). Morphological terminology, as well as the labial tooth row formula (LTRF) follows Altig and Mc Diarmid (1999). The measurements of total length (TL), tail length (TAL) and body length (BL) were taken with a calliper, and the other measurements were taken using a stereo microscope with measuring device and subsequently converted into millimetres. The following further abbreviations were used: BH (body height), BW (body width), TMW (tail muscle width), TMH (tail muscle height), MTH (maximum tail height), TMHM (height of the tail musculature at the midlength of the tail), ED (eye diameter), IOD (inter orbital distance), IND (internarial distance), ODW (oral disc width), TN (number of labial teeth/mm in A2), PN (total number of papillae). A general description of *Mantella* tadpoles is given first, due to their great morphological similarity.

Subsequently the species-specific characters are given separately for each species. Whenever possible only the results for Gosner stages 32–40 were compared. In these stages a developmental "climax" is reached in tadpoles, indicating that they are the best suited for morphological interspecific comparisons (Grosjean 2005).

For the analysis of external morphological characters and oral disc morphology, a table was created using six characters of the external morphology and 23 characters of oral disc morphology. In the table 0 represents an "absence" of the character state in a species and 1 indicates that the character state does apply to the species (table 1). When characters varied within a species, both character states were considered. This table with presence/absence data was used to construct a similarity matrix of tadpoles of all species using Euclidean distances. The similarity matrix was then submitted to Nonmetric Multidimensional Scaling (NMDS) (Guttman 1968; Borg and Lingoes 1987). This is an ordination analysis that produces a bidimensional diagram showing similarities among species. Tadpoles of *M. madagascariensis* and *M. baroni* were excluded from the analysis because of their very advanced Gosner stages and, accordingly, to their non-comparable oral disc morphology. A measure of 'stress' (mismatch between the rank order of distances in the data, and the rank order of distances in the ordination) was calculated. To ensure that the minimum stress function was reached, the NMDS analysis was repeated 10 times with a different position of samples in the initial configuration. The analysis was performed in 2 dimensions.

A second data set containing all morphometric measurements taken from each examined specimen, including number of labial teeth per mm and number of papillae, was divided into three subgroups, partitioned by Gosner stage (GS) (specimens belonging to GS 24–29 group 1, GS 30–39 group 2 and GS 40–44 group 3). A Principal Component Analysis (PCA) was performed for each group. All cases with one or more data missing were excluded from the analysis.

For GS group 3, the analysis was performed using only metric variables, i.e., all variables except TN and PN because these two values could not have been taken due to the very advanced stage of the tadpoles. All statistical analysis were performed using StatSoft, Inc. (2005), STATISTICA (data analysis software system), version 7.1.

2.3 Results

2.3.1 General morphology of tadpoles of the genus Mantella Boulenger

Tadpoles of *Mantella* species share a generally similar morphology, being identical in a variety of characters and divergent in only a few. For a brief morphological comparison of tadpoles of all *Mantella* species examined here, see figures 1–9. All *Mantella* tadpoles can be characterised by a quite uniform colouration. In dorsal view, body ovoid with rounded snout. In lateral view, body is elliptical and snout slopes gently until the oral region and then strongly bends (except in *M. laevigata* whose body is flattened in dorsolateral direction). The ratio BW/BL is very variable, this variability not only being inter- but also intraspecific, and extending between 37–89%. A similar variability is found in IND/IOD which spans between 44–94%. TAL/TL is stable and spans between 49–71%. The external nares are located dorsolaterally, approximately in the middle from snout tip to the eyes. Eyes of moderate size, ED between 6–16% of BL, positioned dorsally, directed dorsolaterally. Spiracle sinistral, inner wall free from the body, with opening positioned laterally, directed posteriorly, visible in dorsal view. Tail fins low, both dorsal and ventral fins approximately of equal height. Caudal musculature well developed, not reaching the tip of the tail. Dorsal fin originates just before the body-tail junction and the ventral fin originates at the posterior ventral terminus of the body. Tail tip slightly rounded (except in *M. viridis* and *M. laevigata* which have pronounced rounded tail tips). Oral disc is elliptical, emarginated (except in *M. laevigata* where it is rounded and not emarginated), positioned anteroventrally. Mouth opens

anteroventrally in *M. pulchra*, *M. betsileo* and *M. bernhardi* and ventrally in *M. aurantiaca*, *M. crocea/milotympanum*, *M. madagascariensis*, *M. viridis* and *M. laevigata*, with an uniserial row of marginal papillae in the lower labium and in the lateral side of upper labium (except in *M. viridis* and *M. bernhardi* that can have either one or two rows and *M. crocea/milotympanum* and *M. laevigata* which have two rows of papillae in the lower labium). Papillae are not pigmented, translucent and conical with rounded tips in *M. aurantiaca*, *M. madagascariensis*, *M. betsileo* and *M. viridis*, and rounded in *M. bernhardi*, *M. crocea/milotympanum*, *M. laevigata* and *M. pulchra*. Upper jaw sheath is concave (except in *M. viridis* in which it is M-shaped) and lower jaw sheath is V-shaped, both finely serrated (except in *M. viridis* and *M. laevigata* where both jaw sheaths have fewer large serrations) and fully black pigmented in more advanced developmental stages (except in *M. aurantiaca*). The size of the jaw sheath is variable; *M. aurantiaca*, *M. betsileo* and *M. bernhardi* have thin, *M. pulchra* and *M. crocea/milotympanum* have middle sized, and *M. laevigata* and *M. viridis* have thick jaw sheaths. Labial tooth row formula of most species is 5(2–5)/3(1) (exceptions are *M. betsileo* 5(2–5)/3, *M. laevigata* 3(2–3)/3(1), in some individuals of *M. crocea/milotympanum* 5(2–5)/3(1–2) and in one individual of *M. aurantiaca* 6(2–6)/3(1)). Characters that differ from this general morphology are given for every species separately below.

The comparison of the characters used in the NMDS analysis is found in table 1 and the results are shown in figure 10.a. The NMDS analysis grouped *M. pulchra* and *M. expectata* very closely, and *M. bernhardi* a bit further apart. Similarly, *M. aurantiaca* and *M. crocea*/*milotympanum* were grouped together. All other species were scattered. The stress value obtained by this analysis was 0.056.

PCA performed separately for specimens in Gosner stage (GS) 24–29 (group 1), GS 30–39 (group 2) and GS 40–44 (group 3) showed slight grouping of specimens within each species. Factor loadings for PCA for all three groups are shown in table 2. For GS 24–29 (group 1; figure 10.b), only specimens of *M. laevigata* are separated, while specimens of *M. pulchra* and

M. bernhardi overlap. For GS 30–39 (group 2; figure 10.c) specimens of *M. viridis* are clearly separated from other specimens (*M. pulchra*, *M. crocea/milotympanum*, *M. bernhardi* and *M. aurantiaca*). For GS 40–44 (group 3; figure 10.d) all specimens that belong to different species are separated from each other (except for a specimen of *M. pulchra* that is positioned very closely to one specimen of *M. crocea/milotympanum*).

Table 1. Morphological characters of tadpoles of the genus *Mantella*. Species abbreviations are as follows: aur = *M. aurantiaca*, croc/milo = *M. crocea/milotympanum*, mada = *M. madagascariensis*, pulc = *M. pulchra*, bets = *M. betsileo*, vir = *M. viridis*, exp = *M. expectata*, baro = *M. baroni*, laev = *M. laevigata*, bern = *M. bernhardi)*.

Species	aur	croc/ milo	mada	pulc	bets	vir	exp*	baro	laev	bern
TAL/TL ≤ 65%	1	1	0	1	0	1	1	0	1	0
TAL/TL > 65%	0	0	1	0	1	0	0	1	0	1
Tail fully pigmented	1	1	0	1	0	1	1	0	0	1
Tail partially pigmented	0	0	1	0	1	0	0	1	1	0
Mouth opens anteroventrally	0	0	0	1	1	0	1	1	0	1
Mouth opens ventrally	1	1	1	0	0	1	0	0	1	0
Oral disc eliptical	1	1	1	1	1	1	1	–	0	1
Oral disc rounded	0	0	0	0	0	0	0	–	1	0
Emarginated	1	1	1	1	1	1	1	–	0	1
Not emarginated	0	0	0	0	0	0	0	–	1	0
Unis. row of marg. pap. in low. lab	1	0	1	1	1	1	1	–	0	1
2 rows of marg. pap. in low. lab	0	1	0	0	0	1	0	–	1	1
Papillae conical	1	0	1	0	1	1	1	–	0	0
Papillae rounded	0	1	0	1	0	0	0	–	1	1
Upper jaw sheath concave	1	1	1	1	1	0	1	–	1	1
Upper jaw sheath M-shape	0	0	0	0	0	1	0	–	0	0
Finely serrated	1	1	–	1	1	0	1	–	0	1
Fewer big serrations	0	0	–	0	0	1	0	–	1	0
Fully pigmented	0	0	–	1	1	1	1	–	1	0
Jaw sheaths thin	1	0	–	0	1	0	0	–	0	1
Jaw sheaths middle sized	0	1	–	1	0	0	1	–	0	0
Jaw sheaths thick	0	0	–	0	0	1	0	–	1	0
Tooth row formula 3(2–3)/3(1)	0	0	–	0	0	0	0	–	1	0
Tooth row formula 4(2–4)/3	0	0	–	0	0	0	0	–	0	0
Tooth row formula 4(2–4)/3(1)	0	0	–	0	0	0	0	–	0	0
Tooth row formula 5(2–5)/3	0	0	–	0	1	0	0	–	0	0
Tooth row formula 5(2–5)/3(1)	1	1	–	1	0	1	1	–	0	1
Tooth row formula 5(2–5)/3(1–2)	0	1	–	0	0	0	0	–	0	0
Tooth row formula 6(2–6)/3(1)	1	0	–	0	0	0	0	–	0	0

*taken from Mercurio and Andreone (2005)

2.3.2 *Mantella aurantiaca Mocquard*

The description is based on a tadpole in Gosner stage 30 from the series of tadpoles catalogued as ZSM 1478/2004 (11 tadpoles) obtained through captive breeding, from parental specimens without precise collecting locality, in 1996–1998 (see Glaw *et al.* 2000) (figure 1).

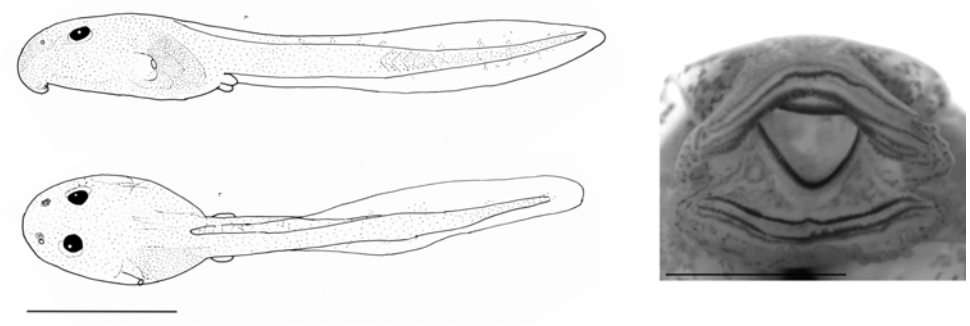

Figure 1. Drawings of the preserved tadpole specimen (GS 30) of *Mantella aurantiaca* (ZSM 1478/2004) in (a) lateral and (b) dorsal view, and (c) photograph of its oral disc. Scale bar represents 5 mm, and 1 mm, respectively.

Table 2. Factor loadings for PCA. GS group 1: Eigenvalue for factor 1 is 10.63 (70.86 % total variance), for factor 2 is 1.60 (10.66 % total variance), for factor 3 is 1.29 (8.61 % total variance). GS group 2. Eigenvalue for factor 1 is 8.38 (57.09 % total variance), for factor 2 is 2.29 (15.26 % total variance), for factor 3 is 1.16 (7.71 % total variance). GS group 3. Eigenvalue for factor 1 is 6.96 (53.50 % total variance), for factor 2 is 1.75 (13.45 % total variance), for factor 3 is 1.20 (9.23 % total variance).

Variable	Factor 1; GS 24–29	Factor 2; GS 24–29	Factor 3; GS 24–29	Factor 1; GS 30–39	Factor 2; GS 30–39	Factor 3; GS 30–39	Factor 1; GS 40–44	Factor 2; GS 40–44	Factor 3; GS 40–44
BL	–0.90	0.14	–0.25	–0.81	0.29	0.03	–0.86	–0.23	0.16
BH	–0.89	0.01	0.10	–0.86	–0.06	0.19	–0.70	–0.37	0.20
BW	–0.94	–0.29	0.14	–0.92	–0.13	0.01	–0.58	0.36	–0.46
TMH	–0.92	0.14	0.26	–0.89	0.02	–0.12	–0.56	0.05	–0.51
TMW	–0.77	–0.40	0.22	–0.91	0.04	–0.07	–0.74	0.56	0.19
MTH	–0.94	–0.15	0.07	–0.91	–0.16	0.12	–0.92	0.13	0.11
TMHM	–0.74	0.13	0.56	–0.74	0.07	0.28	–0.74	–0.11	0.23
ED	–0.95	0.24	0.02	–0.22	0.89	–0.08	–0.67	–0.45	0.24
IOD	–0.95	0.12	0.04	–0.88	0.09	–0.17	–0.26	0.72	0.58
IND	–0.95	–0.19	–0.04	–0.82	–0.25	–0.14	–0.62	–0.36	0.17
ODW	–0.88	–0.20	0.05	–0.69	–0.57	0.00	–0.79	–0.37	–0.18
TAL	–0.71	–0.23	–0.64	–0.71	0.42	–0.12	–0.86	0.29	–0.27
TL	–0.81	–0.10	–0.56	–0.81	0.41	–0.08	–0.93	0.17	–0.18
TN	–0.28	0.90	–0.18	0.44	0.79	0.09			
PN	–0.73	0.51	0.03	0.13	–0.07	–0.97			

The examined specimen had the following measurements: BL 5.7 mm, BH 2.5 mm, BW 3.3 mm, TMH 1.1 mm, TMW 1.2 mm, MTH 2.1 mm, TMHM 0.9 mm, ED 0.6 mm, IOD 1.6 mm, IND 1.3 mm, ODW 1.7 mm, TAL 11.3 mm, TL 17.1 mm, TN 90, PN 40. The mouth opens ventrally. The papillae are conical, uniserial in the lower labium and in the lateral side of upper labium. The jaw sheath is thin, not fully pigmented and finely serrated. The labial tooth row formula is 5(2–5)/3(1). TAL/TL is 67%. Variation (tables 3, 5–7): Average ratio TAL/TL is ≤ 65% and in one specimen LTRF 6(2–6)/3(1) is found.

Table 3. Measurements of tadpoles in GS 24–30 given as means and standard deviation for each species. All measurements given in millimetres. Nr. Spec= number of specimens. .For other abbreviations see table 1 and chapter Materials and methods.

Species	Nr. Spec.	BL	BH	BW	TMH	TMW	MTH	TMHM	ED	IOD	IND	ODW	TAL	TL	TN	PN
		5.7	2.4	3.1	1.0	1.1	1.8	0.8	0.6	1.6	1.3	1.6	10.4	16.3	85	37.5
aur	2	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		0.0	0.2	0.3	0.1	0.1	0.3	0.2	0.0	0.1	0.1	0.11	1.3	1.1	7.1	3.5
		5.6	2.7	3.6	1.3	1.3	2.5	1.0	0.5	1.5	1.2	1.4	8.0	13.5	70	33.7
bern	3	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		0.9	0.3	0.4	0.1	0.1	0.5	0.0	0.0	0.0	0.1	0.1	2.2	2.9	1.5	2.5
		4.4	2.3	2.8	1.0	1.1	2.0	0.7	0.4	1.2		1.4	7.0	11.4		
croc/milo	2	±	±	±	±	±	±	±	±	±	0.7	±	±	±		
		0.7	0.1	0.1	0.3	0.1	0.5	0.0	0.1	0.3		0.0	1.9	2.7		
		4.9	2.4	3.6	1.0	1.2	2.3	0.7	0.4	1.3	1.2	1.5	8.9	13.9	54	23.3
laev	4	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		0.6	0.2	0.5	0.1	0.4	0.3	0.1	0.1	0.3	0.1	0.2	1.6	2.2	1.7	5.0
		7.1	3.5	5.3	1.9	1.9	3.5	1.2	0.8	2.4	1.5	1.9	11.6	18.5	71	37.7
pulc	6	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		0.6	0.5	0.5	0.2	0.2	0.4	0.3	0.1	0.2	0.1	0.1	1.8	2.1	3.6	8.5

2.3.3 Mantella baroni Boulenger

The description is based on a tadpole in Gosner stage 42 from the series of tadpoles catalogued as ZSM 1418/2004 (figure 2) (2 tadpoles). Tadpoles were captive bred in 1996–1998 (see Glaw *et al.* 2000). The examined specimen had the following measurements: BL 7.1 mm, BH 3.7 mm, BW 5.7 mm, TMH 1.8 mm, TMW 1.9 mm, MTH 2.7 mm, TMHM 1.2 mm, ED 1.1 mm, IOD 2.6 mm, IND 1.7 mm, ODW 1.6 mm, TAL 14.8 mm, TL 21.9 mm. The mouth opens anteroventrally. Since all of the tadpoles are already in advanced Gosner stages, the description of the mouth part could not be accomplished. TAL/TL is 68%.

Other tadpoles from the series examined are catalogued as ZSM 1419/2004 (3 tadpoles). All tadpoles were obtained through captive breeding, from parental specimens without precise collecting locality. Variation of all tadpoles is shown in table 6 and 7.

Table 4. Measurements of tadpoles in GS 31–35 given as means and standard deviation for each species. All measurements given in millimetres. Nr. Spec= number of specimens. For other abbreviations see table 1 and chapter Materials and methods.

Species	Nr. Spec.	BL	BH	BW	TMH	TMW	MTH	TMHM	ED	IOD	IND	ODW	TAL	TL	TN	PN
		8.7	3.5	5.2	1.7	2.2	3.4	1.5	0.9	2.3	1.6	2.0	14.5	23.2	72	44.5
bern	8	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		0.8	0.4	0.4	0.1	0.3	0.1	0.2	0.0	0.1	0.1	0.1	1.9	1.1	5.7	6.4
croc/milo	1	7.2	3.3	4.4	1.5	1.5	2.2	1.0	1.0	2.1		1.9	10.1	17.7		
laev	1	7.9	4.4	4.2	1.8	2.3	3.9	1.2	0.7	2.2	1.2	2.2	12.5	20.4	58	33
pulc	1	8.2	3.7	5.6	1.9	2.1	3.6	1.4	0.9	2.6	1.7	2.2	13.6	21.8	70	36
		7.3	3.2	4.6	1.6	1.8	2.9	1.2	0.8	2.2	1.6	2.2	13.3	20.6	63.4	65.6
vir	7	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		0.3	0.2	0.3	0.1	0.2	0.2	0.3	0.1	0.1	0.1	0.3	1.1	1.1	8.5	15.3

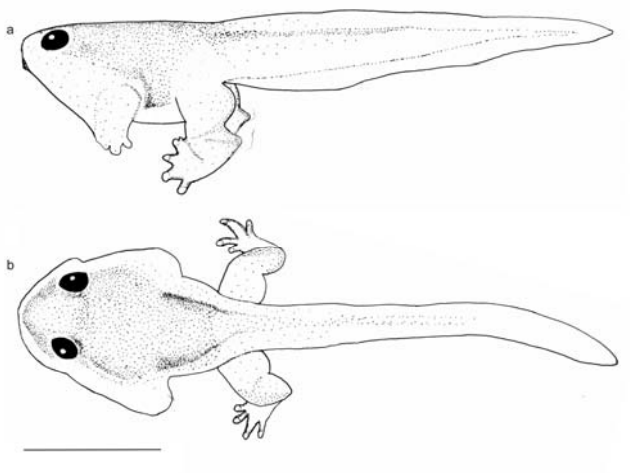

Figure 2. Drawings of the preserved tadpole specimen (GS 42) of *Mantella baroni* (ZSM 1418/2004) in (a) lateral and (b) dorsal view. Scale bar represents 5 mm.

2.3.4 Mantella bernhardi Vences, Glaw, Peyrieras, Böhme and Busse

The description is based on a tadpole in Gosner stage 35 from the series of 7 tadpoles catalogued as ZSM 835/2004, collected by M. Vences on 10 February 2004 in small puddles in a swampy area near a lowland rainforest stream, in Vevembe forest (22°47.686' S, 47°11.228' E, 581 m above sea level) (figure 3). DNA sequence from mitochondrial 16S rRNA gene is deposited in Genbank (accession number FJ830851). The examined specimen had the following measurements: BL 9.2 mm, BH 4.7 mm, BW

5.5 mm, TMH 2.0 mm, TMW 2.3 mm, MTH 4.3 mm, TMHM 1.6 mm, ED 0.9 mm, IOD 2.3 mm, IND 1.6 mm, ODW 2.0 mm, TAL 17.7 mm, TL 26.9 mm, TN 78, PN 47. The mouth opens anteroventrally. The papillae are rounded, uniserial in the lower labium and in the lateral side of upper labium. The jaw sheath is thin, fully pigmented and finely serrated. TAL/TL is 66%. The labial tooth row formula is 5(2–5)/3(1). Variation is shown in tables 3–5, and 7.

Figure 3. Drawings of the preserved tadpole specimen (GS 35) of *Mantella bernhardi* (ZSM 835/2004) in (a) lateral and (b) dorsal view, and (c) photograph of its oral disc. Scale bar represents 5 mm, and 1 mm, respectively.

Table 5. Measurements of tadpoles in GS 36–40 given as means and standard deviation for each species. All measurements given in millimetres. Nr. Spec= number of specimens. For other abbreviations see table 1 and chapter Materials and methods.

Species	Nr. Spec.	BL	BH	BW	TMH	TMW	MTH	TMHM	ED	IOD	IND	ODW	TAL	TL	TN	PN
aur	8	7.0 ± 0.7	2.7 ± 0.2	3.6 ± 0.4	1.4 ± 0.2	1.5 ± 0.1	2.0 ± 0.5	1.0 ± 0.1	1.0 ± 0.1	2.0 ± 0.2	1.4 ± 0.1	1.6 ± 0.2	13.1 ± 1.1	20.1 ± 1.6	86 ± 10.5	51.8 ± 6.4
bern	2	9.0 ± 0.3	4.5 ± 0.3	5.5 ± 0.0	2.0 ± 0.1	2.4 ± 0.1	2.3 ± 0.1	1.8 ± 0.2	1.0 ± 0.1	2.5 ± 0.2	1.7 ± 0.1	2.1 ± 0.1	18.1 ± 0.7	27.1 ± 0.4	78 ± 0.0	46.5 ± 0.7
croc/milo	2	7.0 ± 0.4	3.2 ± 0.1	4.6 ± 0.7	1.9 ± 0.1	2.4 ± 0.2	3.5 ± 0.4	1.7 ± 0.1	1.0 ± 0.1	2.3 ± 0.3	1.6 ± 0.1	2.0 ± 0.1	13.8 ± 2.2	20.7 ± 2.1	72 ± 5.7	56 ± 2.8
laev	1	9.7	4.7	5.4	1.8	2.1	2.3	1.3	1.2	2.5	1.7		12.3	21.9	56	46

Table 6. Measurements of tadpoles in GS 41–44 given as means and standard deviation for each species. All measurements given in millimetres. Nr. Spec= number of specimens. For other abbreviations see table 1 and chapter Materials and methods.

Species	Nr. Spec.	BL	BH	BW	TMH	TMW	MTH	TMHM	ED	IOD	IND	ODW	TAL	TL	TN	PN
aur	1	6.9	2.6	3.9	1.2	1.5		0.9	1.1	2.5	1.3	1.6	12.9	20.2	92	59
		7.6	3.4	5.5	1.8	1.2	2.7	1.4	0.9	2.8	1.5	1.5	15.4	23.0		
bar	5	±	±	±	±	±	±	±	±	±	±	±	±	±	68	70
		0.9	0.4	0.3	0.1	0.2	0.4	0.3	0.2	0.2	0.1	0.1	1.4	1.5		
		10.0	4.8	5.8	2.3	2.2	3.6	2.0	1.3	2.6	1.9	2.5	19.7	29.6		
bets	5	±	±	±	±	±	±	±	±	±	±	±	±	±		
		0.4	0.2	1.2	0.5	0.1	0.6	0.6	0.1	0.2	0.4	0.3	0.5	0.6		
		8.7	3.9	4.5	1.9	2.3	2.0	1.5	1.2	3.4	1.6	1.7	12.8	21.6	63	35.5
croc/milo	6	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		0.5	0.4	0.3	0.2	0.4	1.0	0.7	0.2	0.2	0.1	0.2	3.5	3.2	4.4	0.7
		10.1	4.6	7.0	2.2	3.1	4.6	2.1	1.2	3.8	1.8	2.2	24.1	34.2		
mada	2	±	±	±	±	±	±	±	±	±	±	±	±	±		
		1.0	0.1	0.4	0.1	0.2	0.5	0.6	0.1	0.1	0.0	0.0	1.4	2.3		
pulc	1	8.8	3.1	4.8	1.7	2.0	2.4	1.6	1.4	3.0	1.5	0.8	12.1	21.1		
		7.8	3.1	3.8	1.5	1.7	1.8	1.2	1.1	2.9	1.4		8.9	16.8		
vir	2	±	±	±	±	±	±	±	±	±	±		±	±		
		0.7	0.4	0.5	0.4	0.2	0.0	0.1	0.0	0.3	0.1		2.2	3.1		

2.3.5 Mantella betsileo (Grandidier)

The description is based on a tadpole in Gosner stage 41 catalogued as ZSM 616/2003 (figure 4) (5 tadpoles), collected as embryos in the field from a clutch of a couple of *M. betsileo* in the Forêt de Kirindy/CFPF, and reared in the Kirindy field station, by J. Glos in January 1999. The examined specimen had the following measurements: BL 9.4 mm, BH 4.1 mm, BW 5.8 mm, TMH 3.0 mm, TMW 2.3 mm, MTH 2.6 mm, TMHM 1.8 mm, ED 1.3 mm, IOD 2.5 mm, IND 1.5 mm, ODW 2.4 mm, TAL 19.7 mm, TL 29.1 mm, TN 62, PN 35. The mouth opens anteroventrally. The papillae are conical, uniserial in the lower labium and in the lateral side of upper labium. The jaw sheath is thin, fully pigmented and finely serrated. TAL/TL is 68%. The labial tooth row formula is 5(2–5)/3. Variation is shown in tables 6 and 7.

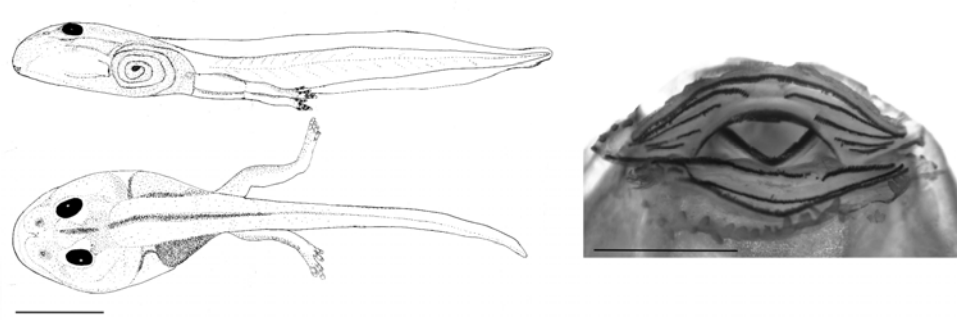

Figure 4. Drawings of the preserved tadpole specimen (GS 41) of *Mantella betsileo* (ZSM 616/2003) in (a) lateral and (b) dorsal view, and (c) photograph of its oral disc. Scale bar represents 5 mm, and 1 mm, respectively.

2.3.6 Mantella crocea Pintak and Böhme × M. milotympanum Staniszewski (F1 hybrid)

The description is based on a tadpole in Gosner stage 36 catalogued as ZSM 1414/2004 (figure 5). Tadpoles from this series are hybrids obtained through captive breeding, from parental specimens collected in 1996–1998 without precise locality (see Glaw *et al.* 2000). The examined specimen had the following measurements: BL 7.2 mm, BH 3.1 mm, BW 5.1 mm, TMH 2.0 mm, TMW 2.6 mm, MTH 3.2 mm, TMHM 1.8 mm, ED 1.0 mm, IOD 2.6 mm, IND 1.7 mm, ODW 2.1 mm, TAL 15.4 mm, TL 22.2 mm, TN 76, PN 54. The mouth opens ventrally. The papillae are rounded, biserial in the lower labium. The jaw sheath is middle sized, partially pigmented and finely serrated. The labial tooth row formula is 5(2–5)/3(1). TAL/TL is 69%. Other tadpoles from the series examined are catalogued as ZSM 1400–1405/2004 and 1408–1415/2004 (altogether 11 tadpoles). Variation is shown in tables 3–7. In some individuals LTRF is 5(2–5)/3(1–2).

Table 7. Mean values and standard deviation of different morphometric ratios for each species of *Mantella* for different Gosner stages. Nr. Spec= number of specimens. For other abbreviations see table 1 and chapter Materials and methods.

GS	Nr. Spec.	Species	BW/ BL	TAL/ TL	ED/ BL	ODW/ BL	ODW/ BW	IND/ IOD	IOD/ BL
24–30	2	aur	0.54 ± 0.05	0.64 ± 0.04	0.10 ± 0.00	0.28 ± 0.02	0.52 ± 0.01	0.78 ± 0.08	0.29 ± 0.01
36–40	8	aur	0.52 ± 0.03	0.65 ± 0.03	0.14 ± 0.01	0.23 ± 0.01	0.44 ± 0.04	0.70 ± 0.08	0.29 ± 0.02
41–44	1	aur	0.56	0.64	0.15	0.24	0.42	0.53	0.36
41–44	5	baro	0.73 ± 0.12	0.67 ± 0.04	0.12 ± 0.03	0.19 ± 0.03	0.26 ± 0.02	0.53 ± 0.07	0.37 ± 0.06
24–30	3	bern	0.65 ± 0.04	0.58 ± 0.04	0.10 ± 0.02	0.26 ± 0.03	0.40 ± 0.03	0.79 ± 0.02	0.27 ± 0.04
31–35	2	bern	0.61 ± 0.11	0.63 ± 0.05	0.10 ± 0.01	0.24 ± 0.03	0.39 ± 0.02	0.71 ± 0.02	0.27 ± 0.02
36–40	2	bern	0.61 ± 0.02	0.67 ± 0.02	0.11 ± 0.01	0.23 ± 0.02	0.37 ± 0.02	0.68± 0.01	0.28 ± 0.03
41–44	5	bets	0.59 ± 0.12	0.66 ± 0.01	0.13 ± 0.01	0.25 ± 0.03	0.45 ± 0.16	0.71 ± 0.13	0.26 ± 0.01
24–30	3	croc/milo	0.65 ± 0.08	0.61 ± 0.03	0.09± 0.04	0.32 ± 0.06	0.49 ± 0.03	0.46	0.29 ± 0.10
31–35	2	croc/milo	0.61	0.57	0.15	0.26	0.43		0.29
36–40	2	croc/milo	0.66 ± 0.07	0.67 ± 0.04	0.14 ± 0.01	0.29 ± 0.00	0.44 ± 0.04	0.68 ± 0.05	0.33 ± 0.03
41–44	6	croc/milo	0.52 ± 0.02	0.58 ± 0.07	0.14 ± 0.02	0.20± 0.03	0.37 ± 0.05	0.48 ± 0.04	0.39 ± 0.02
24–30	4	laev	0.74 ± 0.10	0.64 ± 0.03	0.08 ± 0.01	0.31 ± 0.04	0.41 ± 0.02	0.64 ± 0.43	0.26 ± 0.05
31–35	1	laev	0.53	0.61	0.09	0.27	0.52	0.82	0.28
36–40	1	laev	0.56	0.56	0.12			0.69	0.26
41–44	2	mad	0.70 ± 0.10	0.71 ± 0.01	0.12 ± 0.03	0.22 ± 0.03	0.31 ± 0.01	0.47 ± 0.02	0.37 ± 0.02
24–30	6	pulc	0.75 ± 0.04	0.62 ± 0.04	0.11 ± 0.01	0.27 ± 0.03	0.36 ± 0.03	0.64 ± 0.03	0.34 ± 0.02
31–35	1	pulc	0.69	0.62	0.11	0.27	0.39	0.65	0.32
41–44	1	pulc	0.55	0.58	0.16	0.09	0.16	0.50	0.34
31–35	7	vir	0.63 ± 0.04	0.65 ± 0.02	0.10 ± 0.01	0.30 ± 0.04	0.47 ± 0.05	0.73 ± 0.04	0.30 ± 0.01
41–44	2	vir	0.49 ± 0.01	0.53 ± 0.04	0.14 ± 0.01			0.48 ± 0.01	0.37 ± 0.00

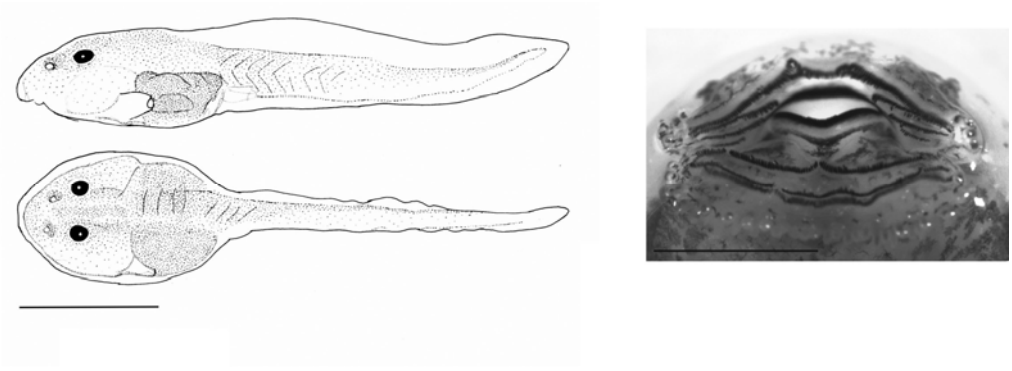

Figure 5. Drawings of the preserved tadpole specimen (GS 36) of *Mantella crocea/milotympanum* (ZSM 1414/2004) in (a) lateral and (b) dorsal view, and (c) photograph of its oral disc. Scale bar represents 5 mm, and 1 mm, respectively.

2.3.7 Mantella laevigata Methuen and Hewitt

The description is based on a tadpole in Gosner stage 25 catalogued as ZSM 1447/2004 (figure 6), obtained through captive breeding, from parental specimens without precise collecting locality, in 1996–1998 (see Glaw *et al.* 2000). The examined specimen had the following measurements: BL 5.2 mm, BH 2.2 mm, BW 3.6 mm, TMH 0.9 mm, TMW 0.8 mm, MTH 2.2 mm, TMHM 0.7 mm, ED 0.4 mm, IOD 1.4 mm, IND 1.1 mm, ODW 1.6 mm, TAL 9.5 mm, TL 14.7 mm. Oral disc morphology is based on a tadpole in Gosner stage 38 catalogued as 1502/2004. Mouth part is not yet fully developed. Body is dorsolaterally flattened, with eyes positioned and directed dorsally. TAL/TL is 65%. The mouth opens ventrally. The mouth part is not emarginated. The papillae are rounded, biserial in the lower labium and in the lateral side of upper labium. The jaw sheath is thick, fully pigmented and with fewer big serrations. The labial tooth row formula is 3(2–3)/3(1).

Other tadpoles examined are catalogued as ZSM 1442–1444/2004, 1502/2004 and 1524/2004 (6 tadpoles). All tadpoles were obtained through captive breeding. Variation is shown in tables 3–5, and 7.

M. laevigata tadpoles examined in this study appear to have unusual oral disc development. The development starts at Gosner stage 25 with the formation of papillae and the first tooth rows. In contrast to the other *Mantella* species, the mouth parts are already considerably degraded at stage 39, with teeth falling out which is possibly a result of earlier metamorphosis, or may be an artefact during captive rearing. Due to a small sample size and lack of specimens captured in the nature, we cannot generalize that this is the case with all *M. laevigata* tadpoles.

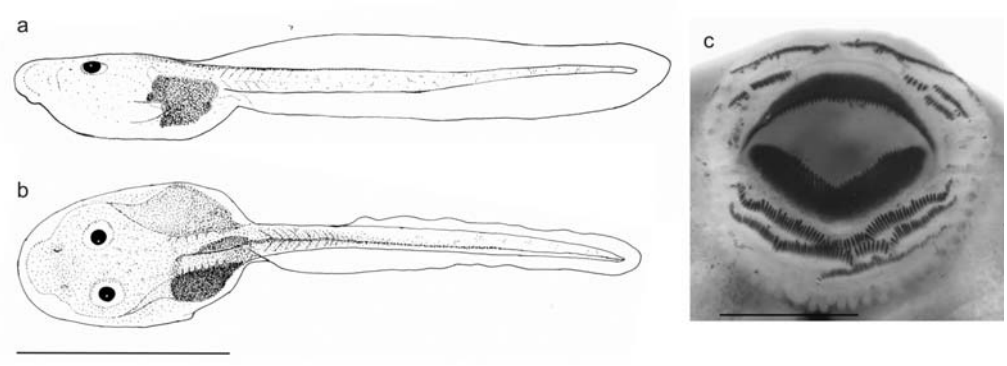

Figure 6. Drawings of the preserved tadpole specimen (GS 25) of *Mantella laevigata* (ZSM 1447/2004) in (a) lateral and (b) dorsal view, and (c) photograph of its oral disc (ZSM 1502/2004; GS 38). Scale bar represents 5 mm, and 1 mm, respectively.

2.3.8 Mantella madagascariensis (Grandidier)

The description is based on two tadpoles in Gosner stages 41 and 42, catalogued as ZSM 1425/2004 (figure 7), obtained through captive breeding, from parental specimens without precise collecting locality, in 1996–1998 (see Glaw *et al.* 2000). The examined specimen had the following measurements: BL 9.4 mm, BH 4.6 mm, BW 7.3 mm, TMH 2.3 mm, TMW 3.3 mm, MTH 5.0 mm, TMHM 2.5 mm, ED 1.3 mm, IOD 3.7 mm, IND 1.8 mm, ODW 2.2 mm, TAL 23.1 mm, TL 32.5 mm. The mouth opens ventrally. The papillae are conical, uniserial in the lower labium and in the lateral side of upper labium. TAL/TL is 71%.

Variation is shown in tables 6 and 7. Since both of the tadpoles are already in advanced Gosner stage, a full description of the mouth part could not be accomplished.

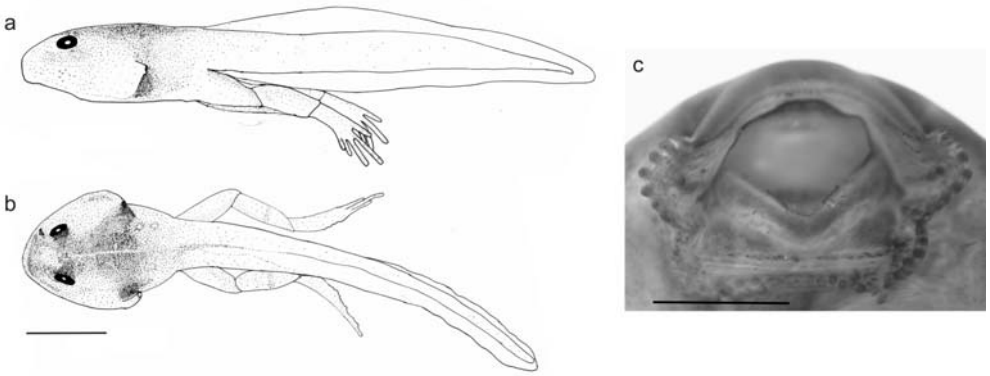

Figure 7. Drawings of the preserved tadpole specimen (GS 41) of *Mantella madagascariensis* (ZSM 1425/2004) in (a) lateral and (b) dorsal view, and (c) photograph of its oral disc. Scale bar represents 5 mm, and 1 mm, respectively.

2.3.9 Mantella pulchra Parker

The description is based on a tadpole in Gosner stage 28 from the series of tadpoles catalogued as ZSM 1/2008 (figure 8) (7 tadpoles), collected at An'Ala forest, on 8 February 2006 by L. Raharivololoniaina and R. D. Randrianiaina. DNA sequence from mitochondrial 16S rRNA gene is deposited in Genbank (accession number FJ830849). The examined specimen had the following measurements: BL 7.1 mm, BH 3.9 mm, BW 5.3 mm, TMH 2.0 mm, TMW 2.0 mm, MTH 3.9 mm, TMHM 1.3 mm, ED 0.8 mm, IOD 2.4 mm, IND 1.6 mm, ODW 1.9 mm, TAL 13.1 mm, TL 20.2 mm, TN 74, PN 50. The mouth opens anteroventrally. The papillae are rounded, uniserial in the lower labium and in the lateral side of upper labium. The jaw sheath is middle sized, fully pigmented and finely serrated. TAL/TL is 65%. The labial tooth row formula is 5(2–5)/3(1). Variation is shown in tables 3–4, and 6–7.

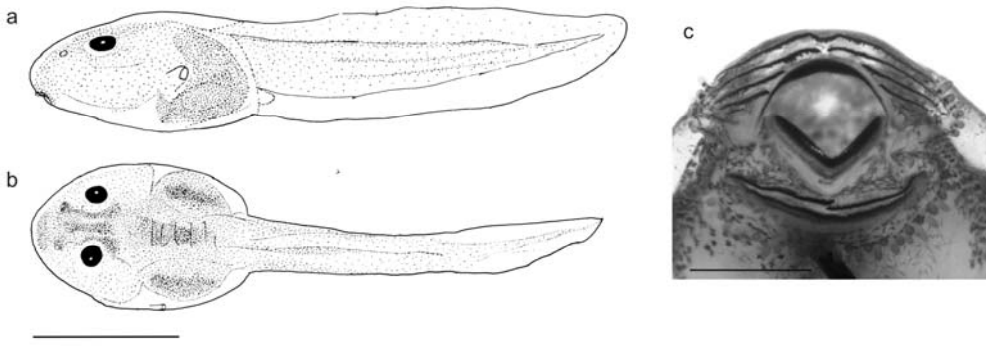

Figure 8. Drawings of the preserved tadpole specimen (GS 28) of *Mantella pulchra* (ZSM 1/2008) in (a) lateral and (b) dorsal view, and (c) photograph of its oral disc Scale bar represents 5 mm, and 1 mm, respectively.

2.3.10 Mantella viridis Pintak and Böhme

The description is based on a tadpole in Gosner stage 34 from the series of tadpoles catalogued as ZSM 797/2004 (figure 9) (3 tadpoles). DNA sequence from mitochondrial 16S rRNA gene is deposited in Genbank (accession number FJ830850). The examined specimen had the following measurements: BL 7.5 mm, BH 3.1 mm, BW 4.6 mm, TMH 1.8 mm, TMW

2.2 mm, MTH 3.2 mm, TMHM 1.4 mm, ED 0.9 mm, IOD 2.3 mm, IND 1.8 mm, ODW 2.0 mm, TAL 11.5 mm, TL 19.1 mm, TN 76, PN 63. The mouth opens ventrally. The papillae are rounded, biserial in the lower labium and in the lateral side of upper labium. The jaw sheath is thick, fully pigmented and with fewer big serrations. The upper jaw sheath has M-shape. TAL/TL is 60%. The labial tooth row formula is 5(2–5)/3(1).

Other tadpoles from the series are catalogued as ZSM 796/2004 (1 tadpole), 798/2004 (3 tadpoles) and ZSM 1574/2004 (2 tadpoles). Tadpoles from series ZSM 796/2004 and 798/2004 were collected by RDR in the field on 20 February 2003 in Montagne des Français, in Madagascar, while tadpoles from the series ZSM 1574/2004 are obtained through captive breeding, from parental specimens without precise collecting locality, in 1996. Variation is shown in tables 4 and 6–7. Some specimens have an uniserial row of marginal papillae.

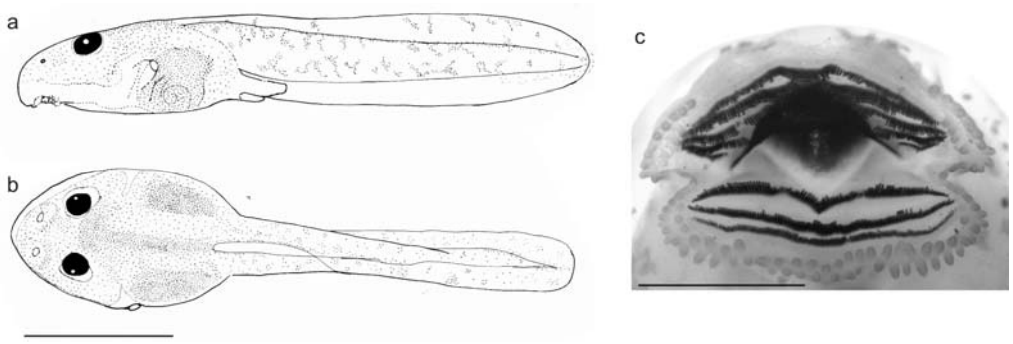

Figure 9. Drawings of the preserved tadpole specimen (GS 34) of *Mantella viridis* (ZSM 797/2004) in (a) lateral and (b) dorsal view, and (c) oral disc. Scale bar represents 5 mm, and 1 mm, respectively.

2.4 Discussion

Mantella have tadpoles of the generalized type (Cannatella 1999), i.e. they do not have any morphological characters showing a high degree of specialization to a specific type of habitat or behaviour, e.g. the specialized funnel-shaped mouth observed in the subgenus *Chonomantis* (genus *Mantidactylus*) (Vences and Glaw 2004), reduced keratinized structures in *Boophis picturatus* as a possible adaptation to sand feeding (Altig and

McDiarmid 2006), or the very prominent oral disc typical for the suctorial stream-living tadpoles in *B. schuboeae* and *B. ankaratra* (Glos *et al.* 2007).

The comparison of the previous description of *M. aurantiaca* with the specimens examined here shows some minor dissimilarities. The papillae of the specimens examined here are uniserial in contrast to the original description of Arnoult (1965), and the labial tooth row formula is variable (5(2–5)/3(1) or 6(2–6)/3(1)). The partial description of *M. laevigata* tadpoles (Glaw and Vences 1992, 1994; Glaw *et al.* 2000) fits the description provided in this study. Tadpoles that were previously described as *M. betsileo* tadpoles are today known to belong to *M. ebenaui* (Glaw and Vences 2007). A rough description given for *M. ebenaui* tadpoles is very similar to that of *M. betsileo*, and only a detailed examination of the former could probably show some differences.

I here described F1 hybrid tadpoles between *M. crocea* and *M. milotympanum* for two reasons: (1) tadpoles of both species were never collected in the field and (2) genetic analyses revealed that these two species are very closely related and might be just colour morphs of a single species (Chiari *et al.* 2004). Their taxonomy is in need of further study (Jovanovic *et al.* 2007).

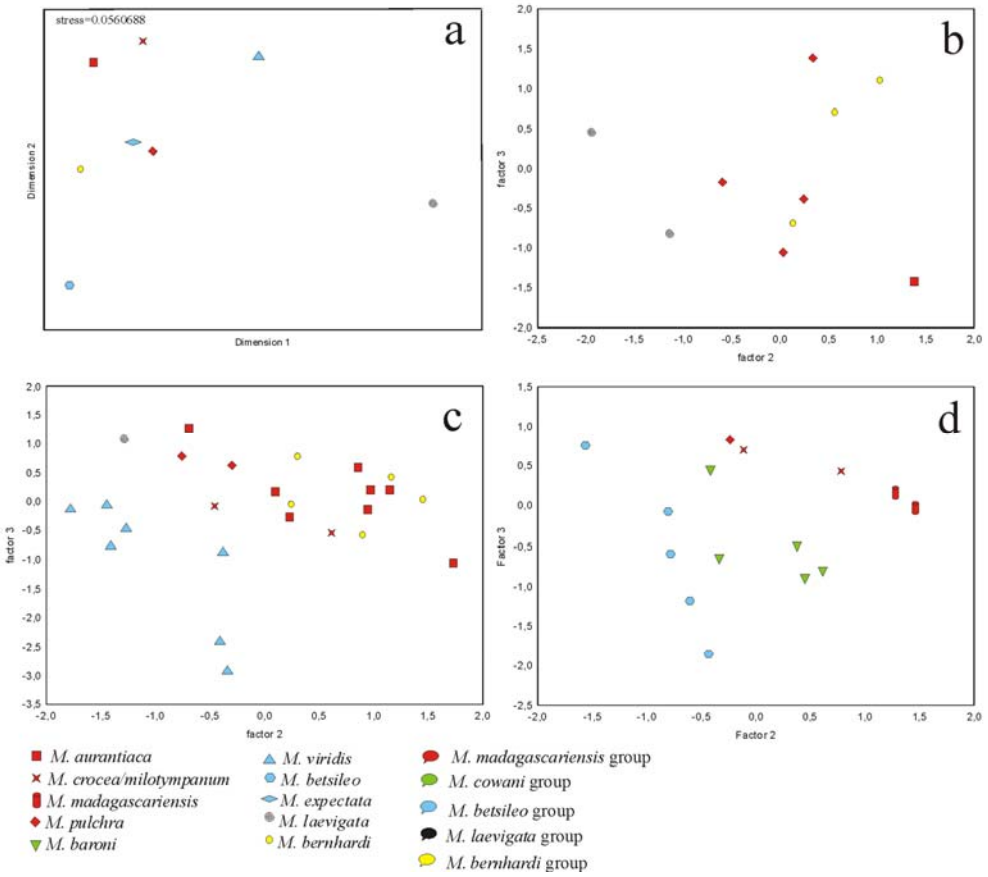

Figure 10. Graphical results obtained by statistical analysis; (a) NMDS analysis performed without *M. baroni* and *M. madagacariensis*; (b) PCA analysis for GS group 1 (includes tadpoles between GS 24–29); (c) PCA analysis for GS group 2 (includes tadpoles between GS 30–39); (d) PCA analysis for GS group 3 (group 3 includes tadpoles between GS 41–44). Each species is represented with its own symbol and each colour represents one *Mantella* species group.

In this comparison I noticed that there is some difference between the tadpoles of different species of *Mantella* when comparing various morphological characters. However, when considering all morphometric measurements, species identification remains difficult. Some morphometric ratios are very variable both intraspecifically as well as interspecifically, such as BW/BL, while some others (e.g. TAL/TL) are stable both intra- and interspecifically and show little variation. Also, LTRF is stable in some species (e.g. *M. betsileo*, *M. laevigata*, *M. pulchra*, *M. viridis*, *M. expectata*) (Mercurio and Andreone 2005) and variable in other (*M. crocea/milotympanum* and *M. aurantiaca*). There is no apparent ontogenetic change of morphology that could be found consistently in all species, i.e. the

ratios of morphological measurements do not change in a predictable manner with increasing developmental stage. Solely, a slight increasing trend is found for ED/BL in all species, and for IOD/BL in *M. aurantiaca*, *M. bernhardi*, *M. crocea/milotympanum* and *M. viridis*, and a slight trend of decrease in BW/BL in *M. bernhardi*, *M. viridis* and *M. pulchra*.

Phylogenetic relatedness might be reflected in morphological similarities, i.e. it could be assumed that closely related species of *Mantella* have more similar tadpoles. The example of *M. crocea/milotympanum* and *M. aurantiaca* shows that this is not a general rule. Both species are very closely related and have morphologically very similar adults (Chiari *et al.* 2004). However, tadpole morphology of these two species does not show such an obvious pattern.

In the NMDS analysis (figure 10.a) based on the absence/presence of various morphological characters (performed without *M. madagascariensis* and *M. baroni* due to their very advanced Gosner stage) only two species are grouped closely together, namely *M. pulchra* and *M. expectata*. This similarity in morphology does not reflect phylogenetic relatedness as both species belong to different species groups. *M. laevigata* tadpoles, that are most deviant from all other *Mantella* tadpoles when inspected visually, showed also in the NMDS the highest dissimilarities to all other species. This is also in agreement with molecular phylogenetic data that place *M. laevigata* together with *M. manery* as a basal group of genus *Mantella* (Chiari *et al.* 2004; Rabemananjara *et al.* 2007). Tadpoles of this species have eyes positioned dorsally, a non-emarginated oral disc and strong jaw sheaths, in contrast to all other *Mantella* tadpoles (except *M. viridis* that also has strong jaw sheaths). Due to the early Gosner stage of the voucher specimen of *M. laevigata*, however, the possibility remains that these differences may be not so obvious in more advanced stages. As well, the unusual shape of the mouth part could be a consequence of an inappropriately applied fixation procedure (e.g. inappropriate handling of specimens, inadequate storage etc). Although it cannot be excluded that the flattened body shape is also a consequence of an inappropriate fixation

procedure, it is together with the dorsal eyes likely an adaptation to the specific habitat niche (Glaw and Vences 1994). While other *Mantella* tadpoles are free living in slow running streams, wetlands or ponds, tadpoles from *M. laevigata* live in phytotelmata (Heying 2001).

Until today, very little is known about natural breeding habitats of *Mantella* species, and in this context most of the tadpoles described here were reared in captivity, without any previous encounters of these tadpoles in the nature. The only tadpoles collected in the field belong to *M. bernhardi, M. pulchra*, *M. betsileo*, *M. viridis* and *M. ebenaui* (previously assigned to *M. betsileo*), and to *M. laevigata*.

Tadpoles of *M. madagascariensis* were never recorded in nature but are presumed to have similar requirements like other species of the *M. madagascariensis* group (swamps). Tadpoles of *M. baroni*, as well as tadpoles of all other species in the *M. cowani* group were also never found in nature due to unknown reasons. In Ranomafana National Park in Madagascar, intensive searches were performed several times (January–February 2007 and 2008) and tadpoles were collected from many streams where adult individuals of *M. baroni* were common but none of the tadpoles of this species were found. In this case, I presume that some systematic omission has happened. The possibility of searching in the wrong period of the year can most certainly be excluded because the animals were calling at the researched sites, as well as the possibility of direct development (when bred in captivity, they do have tadpoles).

The PCA showed species specific morphological separation of *Mantella* tadpoles, and it is more pronounced in tadpoles of more advanced Gosner stages (keeping in mind that for different stages different species were used). Since PC factor 1 for all three GS groups was mainly contributed by size related variables, it was therefore omitted from the interpretation. In the analysis for GS group 3, we can see that PC factor 2 (mainly IOD) very clearly separates specimens of *M. betsileo* from other species. Both IOD (2.38–2.86 mm) values as well as IOD/BL (0.24–0.28) are very stable, while

in other species both of these values are very variable (in most of the species IOD being greater than in *M. betsileo*).

Intraspecific morphological variability of *Mantella* tadpoles as it was found in this study can be a result of several influences, such as genetic background and environmental factors. Intraspecific variability in tadpoles reared in captivity can be caused by genetic factors. The origin of many of the specimens used in this study is unknown as they were obtained through the pet trade, but the same individuals have been used for genetic analyses (e.g. Schaefer *et al.* 2002) and it is unlikely that any of them had a genetically divergent background, e.g., originating from geographically distant populations. On the other hand, specimens collected in the field can show morphological variability either as a result of genetic variability or the ability to exhibit phenotypic plasticity. Taken in consideration general variability in *Mantella* tadpoles, we can examine the argument of Wilbur and Collins (1973) who have proposed that amphibian larvae might respond adaptively to changes in their environment. Phenotypically plastic responses to environmental change are typically compartmentalized by the type of environmental cues that cause the induction. In amphibian larvae for example, it can be influenced by temperature (Harkey and Semlitsch 1988; Newman 1998), but different types of environmentally induced responses might very well be related to each other. Additionally, factors that account for differences in growth rate and size at metamorphosis, are shown to have effects on the oral structure in *R. temporaria* larvae (Vences *et al.* 2002). In this study relatively high variability by PCA between the specimens of *M. bernhardi*, *M. aurantiaca* and *M. pulchra* was observed.

Tadpoles of the genus *Mantella* do not appear to bear many useful characters for determining phylogeny. Also *Mantella* adults appear morphologically very homogeneous, both in terms of morphology and ecology. Likewise, there is no great divergence in tadpole morphology, although some differences exist. We thus may hypothesize that ecological factors in *Mantella* species have stronger influence on tadpole morphology than does the phylogeny.

3 Skeletochronological analysis of longevity in Malagasy poisonous frogs, genus *Mantella*

Abstract

Longevity of five species of endemic Malagasy frogs of the genus *Mantella* (*M. aurantiaca*, *M. baroni*, *M. bernhardi*, *M. crocea*, *M. madagascariensis*) was examined applying skeletochronology. Among several other methods to assess the age of amphibians, this has emerged as one of the most reliable ones. In total 96 specimens from nine different localities in Madagascar were analyzed (Ampangadimbolana, Anosibe An'ala, Besariaka, Manombo two sites, Ranomafana-Ranomafanakely, Torotorofotsy two sites, Vevembe). In this study I confirm the short longevity of *Mantella* frogs. In more than half of the specimens (57%), no lines of arrested growth (LAGs) were found, and the number of LAGs recognized in the remaining specimens ranged between zero and two.

Keywords: skeletochronology, Amphibia: Mantellidae, Madagascar, *M. aurantiaca*, *M. baroni*, *M. bernhardi*, *M. crocea*, *M. madagascariensis*

3.1 Introduction

Poisonous frogs from genus *Mantella* are attractive, small diurnal frogs. They are highly priced in pet trade, particularly the more brilliantly coloured species (e.g. *M. aurantiaca*), such that large numbers of specimens are exported from Madagascar every year (Behra 1993; Rabemananjara *et al*. 2008b). In a concerted effort to monitor the trade, all *Mantella* species have been placed on Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Vences *et al*. 2004; Andreone *et al*. 2006). Despite of their commercial interest and the fact that many publications are available on the husbandry of most of the species, very few data on their longevity in natural environment are available, namely just for *M. cowani*, *M. baroni* (Guarino *et al.* 2008) and *M. expectata* (Guarino *et al*. in review). In all three species of *Mantella*, the youngest specimens studied were less then one year, and the oldest up to three years old.

Age determination is crucial to investigation of longevity, population dynamics, fecundity, and a variety of other biological studies. The ages of adult amphibians cannot be precisely estimated on the basis of body length or mass, because body size is strongly affected by the environmental parameters and genetic predisposition (Rozenblut and Ogielska 2005). Several methods are known to assess the age determination of amphibians and reptiles. To date, these are eye lens weight, testis lobation, size-frequency data, mark-release recapture and skeletochronology (Castanet and Smirina 1990). Skeletochronology has emerged as the most reliable and powerful tool to estimate the age and longevity of amphibians and today is routinely used (e.g. Castanet *et al.* 1996; Kumbar and Pancharatna 2001; Measey 2001; Sinsch *et al.* 2001; 2002; Bovero *et al.* 2006). The basis for skeletochronology are lines of arrested growth (LAGs) found in the bones of amphibians and reptiles. LAGs correspond to resting periods in amphibian hard tissues. During hibernation, bone tissue apposition stops and a LAG is formed; therefore, each LAG represents 1 year of an anuran's life, as was

confirmed by mark-recapture studies (Rozenblut and Ogielska 2005). LAG formation can reflect not only seasonal, but also intrinsic biological rhythms, such as one might encounter in tropical species that are active year-round without hibernation (Guarino *et al*. 1998; Kumbar and Pancharatna 2001; 2002). The most useful skeletal elements for these studies are long bones, which increase in thickness (radial growth) as the result of centripetal apposition of new tissue, such that the most external layer is the youngest.

In this study I present longevity data for five species of *Mantella* (*M. baroni*, *M. bernhardi*, *M. madagascariensis*, *M. aurantiaca* and *M. crocea*) obtained by skeletochronology.

3.2 Materials and methods

3.2.1 Collection of specimens

111 mantellid frogs of five species were analyzed: *M. baroni* (N=20), *M. bernhardi* (N=46), *M. madagascariensis* (N=15), *M. aurantiaca* (N=29) and *M. crocea* (N=1). Specimens were collected in 2004 at nine different locations, and therefore, nine different populations were examined. All frogs were sampled by opportunistic searching that involved observation and removal of leaf litter and low vegetation or by precisely targeting calling males, especially in the case of *M. baroni.* All frogs were collected on relatively small plots of a maximum of 0.5 ha, often much smaller. Individual frogs were sexed and measured for snout to vent length (SVL) to the nearest 0.1 mm. Frogs were collected in Ranomafana National Park in southeastern Madagascar in a site locally known as "Ranomafanakely" (along National Road 45 from the village of Vohiparara toward the town of Fianarantsoa), in Besariaka (located to the north of Ranomafana), Manombo and Vevembe (both located far to the south). At two of these sites, *M. baroni* and *M. madagascariensis* were collected in syntopy. At Ranomafana, *M. madagascariensis* was found close to the road in a tiny patch of degraded

forest dominated by *Eucalyptus* spp., whereas *M. baroni* was found at a distance of less than 50 m in primary rainforest. At Besariaka, the two species were fully mixed and using exactly the same microhabitat. List of frogs sampled at each location is provided in Appendix, table 1 together with GPS coordinates and voucher identification numbers. Voucher specimens are deposited at the Zoological Museum Amsterdam, the Université d'Antananarivo, Département de Biologie Animale (UADBA), and the Zoologische Staatssammlung München. The same *Mantella* specimens were used for alkaloid content analyses by Daly *et al*. (2008) and data are available for further comparison.

3.2.2 Skeletochronological analyses

Skeletochronology was performed applying following procedure: bones of the forelimbs (humerus and radio-ulna, and in some cases phalanges) were first decalcified in 3% HCOOH (time of decalcification was variable, from 2-20 min depending on the size of the bones). Afterwards, the bones were rinsed in deionised water for 10 minutes. Samples were then mounted in tissue-tek on cryomicrotome and sectioned (25-30 micrometer thickness). Sections were collected in 1xPBE buffer and spread on the chromalaum/gelatin-coated glass slides (in order to attach to the slide). Sections were dried for few hours and stained in 0.05% cresyl-violet, followed by rinsing in deionised water for few minutes. Stained sections were left to dry for ca half an hour and fixed. Sections were checked on the light microscope, and photographed with Nikon Coolpix 595 digital camera. Before sectioning bones of *Mantella*, the method was first tested on the European amphibian species *Rana dalmatina* and *Triturus cristatus*.

3.2.3 Statistical analyses

Number of LAGs determined by skeletochronological analyses was compared applying Spearman Rank Order Correlations with SVL and

amount of alkaloids of each individual (based upon total alkaloid compounds ion chromatogram intensities with 10^4 or greater = major, 10^3 – 10^4 = minor, $\leq 10^3$ = trace), using STATISTICA 7.1. (data analysis software system; StatSoft, Inc. 2005). Due to a small number of samples of each species, the analyses were performed by merging all species together.

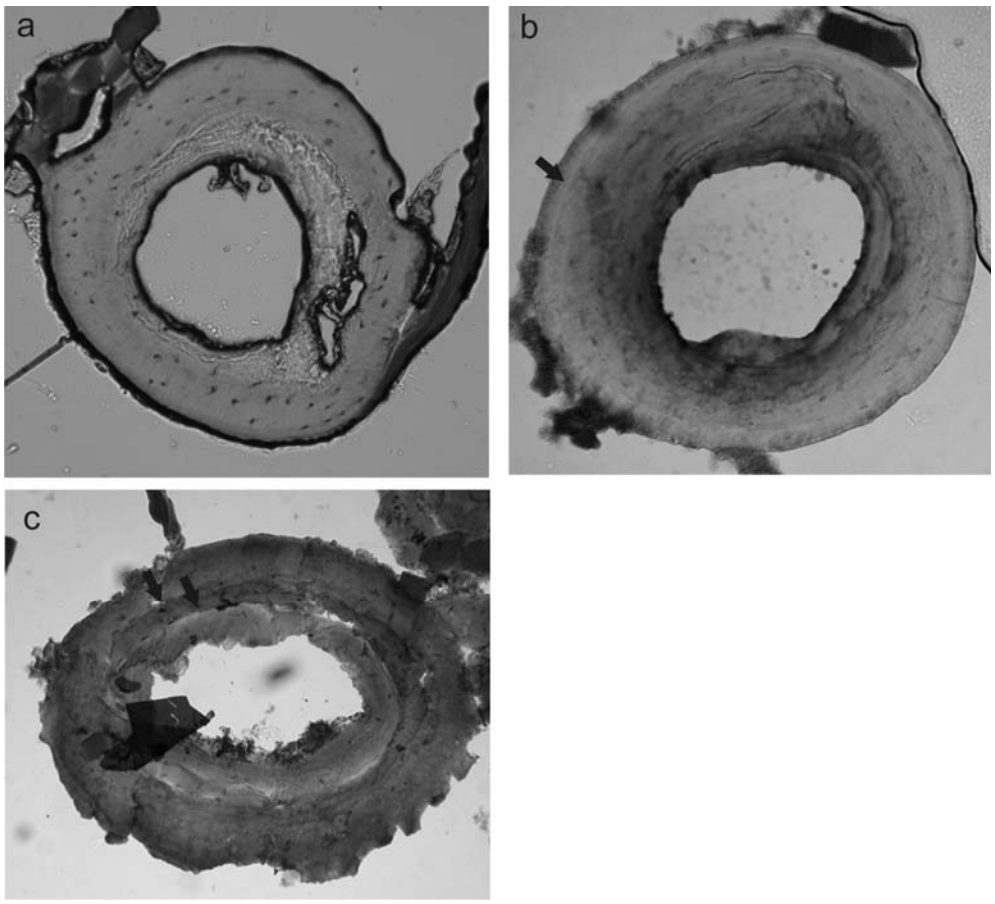

Figure 1. Representative humerus cross-sections. Arrows indicate lines of arrested growth. a) No LAGs present; *M. crocea*, SVL 17.7 mm; b) one LAG present; *M. aurantiaca*, SVL 20.2 mm; c) two LAGs present; *M. madagascariensis*, SVL 24.9 mm.

3.3 Results

In adults of all *Mantella* long bones cross sections at diaphyseal level were composed of two concentric layers, mostly not vascularised. Periosteal bone comprises the outermost layer and was more developed with mostly parallel fibered bone, and the endosteal bone encircles the medullar cavity. The two layers were sometimes very similar in texture, disabling the discrimination

of endosteal from periosteal bone. For this reason, the whole bones were sectioned in order to get more reliable results.

Out of 111 samples that were processed, 96 sections showed recognizable bone structures. I did not obtain any precise sections in 18 samples to be able to determine the age of the frog and left them out of further analyses. Results for each species are shown in table 1. Surprisingly, in 55 samples (57%) I could not recognize any LAGs, in 38 samples (40%) one LAG was recognized and in only three samples (3%) two LAGs were recognized (table 2). Representative sections are shown in figure 1.

Table 1. Number of samples of each species for which sections with recognizable bone structures were obtained.

Species	Number of samples	No LAGs	1 LAG	2 LAGs
M. aurantiaca	25	9	15	1
M. baroni	17	15	1	1
M. bernhardi	43	25	18	0
M. crocea	1	1	0	0
M. madagascariensis	10	5	4	1
Total	96	55	38	3

Comparing SVL and number of LAGs showed that there is no correlation between these two variables, when taking in consideration all the results (including sections that did not have any LAGs; r=0.09, p= 0.37). However, including only those samples with at least one LAG resulted in a positive correlation between SVL and number of LAGs (N= 41, r= 0.33, t= 2.20, p= 0.03). Comparison of amount of alkaloids and number of LAGs and did not show any correlation.

Table 2. SVL data for the specimens with two LAGs in comparison to the mean SVL and SD values of the specimens with one LAG, for each of these species.

Species	SVL (mm)	Mean SVL ± SD (mm)
M. aurantiaca	21.0	21.19 ± 1.93
M. baroni	26.0	26.2
M. madagascariensis	24.9	22.95 ± 1.67

3.4 Discussion

I have confirmed in this study that LAGs are present in the long bones of *Mantella* frogs. In *M. crocea*, I found no LAGs present, but this result should not be generalized for this species because only bones from one specimen are examined. In other four species studied (*M. baroni*, *M. bernhardi*, *M. madagascariensis*, *M. aurantiaca*), I found at least one specimen with one LAG present, and in three of these species (*M. baroni*, *M. madagascariensis*, *M. aurantiaca*), one specimen each with two LAGs was found.

Our samples were cut in several series and sections obtained in the last two series did not show to be as good as those from previous series. In these sections fewer LAGs were found then in previous series and LAGs could not be distinguished as easy as in the previous sections. This could be due to a methodological problem, although the same protocol was used in both cases, including some variations in the second case. Alternatively, LAGs can be absent in the populations with constant, year-round activity, as it was found in some of the tropical frogs including one population of a species from Madagascar (*Dyscophus antongili*; Tessa *et al*. 2007) and two Indonesian species (*Fejervarya limnocharis* and *F. cancrivora*; Kusrini and Alford 2006). At first glance, this explanation could not be applied to *Mantella* since they are generally known to hibernate between May and August (Rabemananjara *et al*. 2008a). On the other hand, all specimens from Ranomafana National Park, both *M. baroni* and *M. madagascariensis*, did not show any LAG formation. Despite the fact that there is a seasonal difference between the temperature and rainfall in Ranomafana, there is no strictly dry and rainy season, and the rain falls on average on 200 days per year (Andreone 1994). This could allow longer activity periods for *Mantella* frogs. Although this lack of seasonality does not have to cause complete loss of hibernation, it can postpone it or intercept it, so that there is no clearly distinguished hibernation. *Mantella* are generally found in the rainy season, when they have the annual activity peak, and when they breed. Both *Mantella* species found in Ranomafana (*M. baroni* and *M.*

madagascariensis) were never encountered during the "dry" season. Apart for the hibernation, another possibility is that the search was not comprehensive enough. They live in high grass and small bushes, and because of good hiding opportunities in this habitat type, they are mostly found only when calling. The possibility of their occasional activity during this time of the year cannot be excluded, and this could influence the formation of LAGs and explain the results for this site.

Similarly to my case with more then 50% of specimens without LAGs, was also found by Guarino *et al.* (in review) in *S. gottlebei*; nearly 62% (8 of 13) of males and 32% (8 of 25) of females did not show any LAGs. *S. gottlebei* lives in pronounced seasonal conditions, but the authors argue that the canyon microhabitat does not undergo such extreme changes in the weather conditions as does the whole habitat.

Our longevity data slightly differ from data obtained for other *Mantella* species; Guarino *et al.* (2008; in review) found in some specimens of *M. cowani*, *M. baroni* and *M. expectata* three LAGs. It needs to be stressed that their original sample size was greater than ours (41 specimens *M. cowani*, 42 *M. baroni* and 63 *M. expectata*), but the number of specimens from which they got good sections was only slightly higher then ours in *M. cowani* (26 specimens) and *M. baroni* (24 specimens).

Mantella are captive bred for several decades, and there are numerous data on their breeding behaviour and clutch size, as well as the time to metamorphosis in captivity. Additionally there are many popular articles and terrarium keeping guides for *Mantella* (e.g. Glaw *et al.* 1998; 2000; Rabemananjara *et al.* 1996; Staniszewski 1997). But despite all that, there are very few records on *Mantella* longevity in captivity. Staniszewski (1997; 2001) reports the oldest specimen of *M. aurantiaca* being twelve years old. Apart from that, the average life span for all *Mantella* species in captivity is considered to be between five to eight years. Due to a great difference between captivity and natural habitat conditions (such as food availability and predation) longevity data from captivity cannot be compared to longevity data obtained from wild populations.

Guarino *et al.* (2008) put forward the hypothesis that smaller species have shorter life span; this is also confirmed in my case in *M. bernhardi,* the smallest species of *Mantella*. Despite the biggest sample size, Ifound no specimens of *M. bernhardi* having two LAGs. Overall, existing data on longevity of *Mantella* frogs in natural habitats showed maximum of three years in all *Mantella* species previously examined (*M. cowani*, *M. baroni* and *M. expectata*). Furthermore, life span is documented for some *Mantella* species in captivity (Staniszewski 1997; 2001). In fact, Staniszewski (2001) referred to a maximum longevity of 7 years in *M. cowani* but these data cannot be compared and applied to wild populations.

LAGs were visible in all but one species that we studied, and this confirms that skeletochronology is a valid method in age determination of *Mantella* frogs. Nevertheless, in some specimens LAGs were visible and distinct, while in some populations bone structures were much less obvious. This may be due to some procedural aspects, such as reduced affinity to the dye, and histological characteristics of the bone. Different protocols were used for both, decalcification and staining, but gave similar results. The problem could be methodological, but more likely is related to life history traits.

The short life span observed in all *Mantella* species studied until now, is most likely in relation with the small size they reach. Usually, they do not grow more then 30 mm in SVL. The life span of frogs varies depending on the species but the smallest ones are also the short-lived species (Guarino *et al.* 2008).

3.4.1 Skeletochronology as an age estimation method

Although skeletochronology has been recognized as a reliable method for age determination in amphibians and reptiles, there are several points that need to be taken into account. One of the most important aspects is the selection of the bones. In general, long bones of the limbs, especially the middle part of diaphysis where the periostal cortex is the thickest and the

medullar cavity the narrowest, are considered to be the most appropriate for this type of analysis due to the similar patter of differentiation (Castanet and Smirina 1990; Rozenblut and Ogielska 2005). Moreover, in age determination from phalanges, it is necessary to discard the last phalanx (with the claw) where layers are not visible. Castanet and Smirina (1990) suggest the use of dry bones as the most expedient. They also emphasize that even if a correspondence between the number of layers in bones and body size is found in many species of amphibians and reptiles, there are no strict and necessary correlation between the two variables. As a rule, the largest individuals are not necessarily the oldest, and individuals growing slowly and gradually are generally those who live longer. The general layering pattern in different bones of an individual is usually the same but different bones and even different parts of the bone, could differ in duration of their growth periods and hence record different numbers of growth cycles. Even with bone preparations of good histological quality it is rarely possible to determine age just by counting the rest lines present in one section.

3.4.2 Conservation

Assessing the age structure of *Mantella* frogs is important for managing the pet trade in sustainable manner. Unfortunately, the longevity data alone are not sufficient to enable the calculation of annual collection quotas for different *Mantella* species. To give precise estimations, we need much more information on the natural history of *Mantella*, such as population size (available for some species, e.g. Andreone *et al*. 2006; Vences *et al.* 2008; Vieites *et al.* 2005), number of eggs per clutch (many data available, although mostly from captive bred specimens, e.g. Glaw *et al.* 2000; Guarino *et al.* 2008, Staniszewski 1997; 2001), tadpoles survival rate (unfortunately not yet studied, but very important). Additionally, these data are becoming even more important in the context of habitat loss because

many of *Mantella* species live in areas that are not protected and are very threatened by “tavy”, local slash and burn practise.

4 Examining the effectiveness of aposematic colouration in Malagasy poisonous frogs, genus *Mantella*

Abstract

The effectiveness of aposematic colouration in Madagascan poison frogs, genus *Mantella* was experimentally tested exposing clay frog models to rainforest habitats in two areas of Madagascar. The models had different colours (brown, resembling any non-conspicuous frogs; orange, resembling *Mantella aurantiaca*; and black-yellow, resembling *Mantella baroni* / *M. madagascariensis*). All three types of models were exposed in an area populated by *Mantella aurantiaca* (Torotorofotsy wetland) and in an area populated by the externally similar *M. baroni* and *M. madagascariensis* (Ranomafana National Park). In both areas I studied plots populated by *Mantella* as well as control plots where these frogs do not occur. Altogether 2506 clay models were set in the field. Predation observed on models was tested for correlation between colouration of models as a distinctive predictor of predation, time of predation (day, night), shape (frog, shapeless), position of the model, influence of predators' experience, as well as learning effect among predators.

I found only one factor influencing the predation in Andasibe in *Mantella* plot both in 2007 and in 2008, the position of the model (on/ above the ground). All the other predictors showed not to be significant determinants of predation. Additionally, since only very small percent of predation was by birds, we can presume that these types of experiments are not appropriate for studies of aposematism of *Mantella* frogs.

Keywords: Amphibia: Mantellidae, Madagascar, aposematism, predation experiments, *Mantella aurantiaca*, *M. baroni*, *M. madagascariensis*

4.1 Introduction

Aposematism is the association, in potential prey organism, of the presence of a warning signal (most commonly bright colouration) with unprofitability to predators (Cott 1940; Edmunds 1974; Guilford 1990). Theoretical model involving learning psychology has suggested how warning coloration in unprofitable prey could evolve and become stable. This model shows that potential predators learn to avoid noxious prey more readily when those preys have conspicuous colour pattern.

High morphological convergence which also extends to coloration makes anurans well suited organisms for studies of vertebrate evolutionary patterns, such as aposematism. Most frogs are rather cryptic and only a few groups can be characterized as genuinely aposematic (Santos *et al*. 2003; Vences *et al*. 2003; Darst *et al*. 2006).

Only few data exist about predation on poisonous frogs in general. There are in total around 40 reports published on predation on poisonous frogs, e.g. Guimaraes *et al*. (2004), Cuello *et al*. (2005), Menin (2005), Smith and Green (2005); ten of them refer to frogs from family Dendrobatidae (Daly and Myers 1967; Brodie and Tumbarello 1978; Myers *et al*. 1978; Fritz *et al*. 1981; Szelistowski 1985; Hedstrom and Bolanos 1986; Master 1998; Master 1999; Summers 1999; Gray *et al*. 2002). Until now, there are only four published records on predation on *Mantella* frogs; two by Heying (2001) and two presented in Chapter 6. A few feeding experiments were carried out on dendrobatid frogs and naïve avian predators (domestic chicken) to assess learning abilities (speed of avoidance learning and degree of avoidance after learning, and stimulus generalization; Darst and Cummings 2006; Darst *et al*. 2006).

Here I present data of a survey of the efficiency of aposematic colouration of Malagasy poison frogs by clay model experiments performed in their natural habitats. Clay model experiments have been extensively used for studies of aposematism in amphibians and reptiles in several previous studies with snakes, lizards and salamanders (e.g. Brodie 1993; Brodie and

Moore 1995; Hinman *et al*. 1997; Castilla and Labra 1998; Pfennig *et al*. 2001; Wüster *et al*. 2004; Kuchta 2005; Kuchta *et al*. 2008), but only a few studies have been conducted using frog models (e.g. Darst, pers. comm.; Saporito *et al*. 2007; Noonan and Comeault 2009; Anderson 2006). Usually, these experiments obtained reliable indications on the preference or avoidance of certain type of prey.

Our experiments refer to the three species of *Mantella*- *M. aurantiaca*, *M. baroni* and *M. madagascariensis*. *M. aurantiaca* is distributed in the central eastern Madagascar (Bora *et al*. 2008), *M. baroni* population stretch from central east further to the south, to Ranomafana National park, and *M. madagacariensis* is found in the central-east and in Ranomafana (Jovanovic *et al*. 2007). All *Mantella* species have a very patchy distribution while the latter two species live in some areas in syntopy. Additionally, these two species represent an example of Müllerian mimicry, a phenomenon of the close similarity of coexisting unpalatable prey species (Schaefer *et al*. 2002).

In these experiments I was testing if there is a difference between predation on different colours of models, and if predation was different in plots with naive, or with experienced predators. Ipresumed naive predators not to be choosy towards different colours of prey, but experienced predators to avoid prey of the coloration they have encountered before as non-edible.

4.2 Materials and methods

4.2.1 Clay model experiments

Frog models were made using non-toxic clay produced by Eberhard Faber (Art. Nr. 8421-1 yellow, -4 orange, -7 brown, -9 black) by pressing it into silicone molds made upon *Mantella madagascariensis* carcass. Models were made in three different combinations of colour - brown colour resembling any non-conspicuous frog, orange colour resembling *M. aurantiaca* and

black-yellow colour resembling *M. madagascariensis* / *M. baroni* (Figure 1).

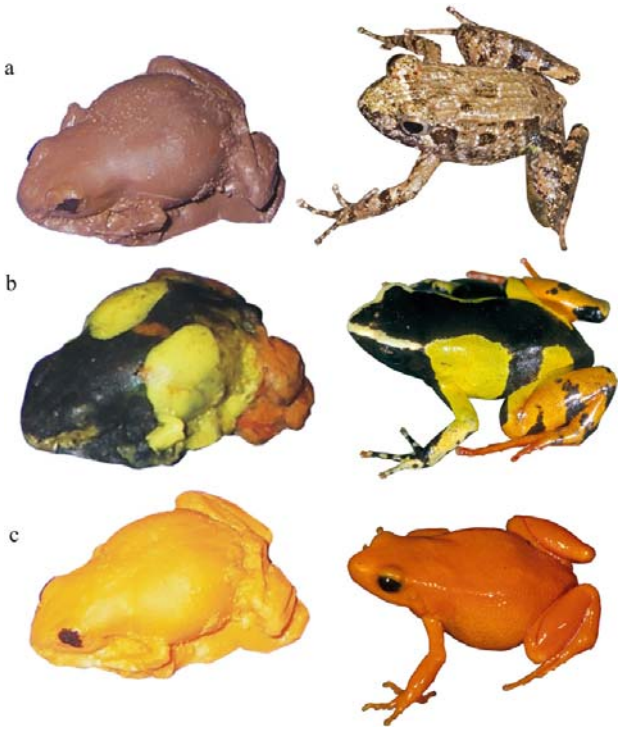

Figure 1. Frog clay models and the "original" frogs: a) non conspicuous frog, b) *M. baroni*/*M. madagascariensis*, c) *M. aurantiaca*

As a negative control, clay balls in the same colour combinations were used ("shapeless" models; except in the Andasibe region in 2007). All types of models were set randomly on a transect, at the same time, in the field. Models were set in different habitat types (secondary and eucalyptus forest, marsh, surrounding of the small streams and ponds) and depending of the habitat type, they were placed on the ground, on the leaf litter, moss, dead fallen trees, on leaves of *Pandanus* plants, within 3-5 m distance.

4.2.2 Experiments preparation

In order to be able to design the experiment in a way that it is likely to give measurable results in Madagascar, preliminary experiments were carried out in the Schapen forest near Braunschweig in September 2006. Models were

set in two series; the first included brown as cryptic models and several colour combinations as aposematic, while the second included only black-yellow and orange models as aposematic. During both preliminary experiments, diurnal and nocturnal predation was observed separately. Data obtained from both series were partially analyzed together.

Prior to the experiments in Madagascar, several species of *Mantella* (*M. aurantiaca*, *M. expectata*, *M. crocea*, *M. baroni*, *M. madagascariensis* and *M. laevigata*), as well as clay used for models, were examined for UV reflectance by taking black and white photos (KODAK BW400CN) with analogue camera Minolta SRT 101 through UV transmission filter (Schott and Gen. Mainz, UG 11, 330-350 nm, 5x5cm, 2 mm thick) which excludes all but UV light. Photos were taken in diffusely lighted photo-box under standardized light conditions. Light sources comprised two Kaiser 5454 daylight lamps producing colour temperature of 5400K. Since both, *Mantella* frogs and clay showed no UV reflectance, colours for models were chosen by eye.

Additionally, in order to exclude possible olfactory preference for a specific colour of clay, diurnal and nocturnal predation on the clay balls in the same colour combinations were examined in the laboratory, using four mice (*Mus musculus*) as predators. Since it showed no significant preference for a specific clay colour, we will ignore the possibility of predator attraction by olfactory signals.

Experiments in Madagascar were carried out between January and March in 2007 and in 2008. At the beginning of the second season of the experiments, models were checked for predation two times a day: early in the morning, and in the afternoon, in order to separate diurnal from nocturnal predation. After the first week, and during the first season of the experiments, models were checked several times in the subsequent three to four weeks. As predation Iconsidered displacement of the models, bite marks and models being totally destroyed by predators, in the time period between two surveys. Multiple bite marks between two surveys were considered as one predation. Some of the bite marks were assigned to

probable predators, i.e., small mammals vs. birds, but unfortunately, for most of the marks, a reliable identification of predator was not possible. Predated models were replaced with new models in the same colour combination. In 2007 missing models were initially recorded as predated. In order to exclude the possibility that they were not predated but simply not found among the leaf litter, these data were analyzed both with missing models as predated and as void data. In 2008 missing models were ignored and treated as void data. Exact location of each model was recorded and marked with an associated number using brown tape, approximately 1-1.5m above the ground on trees.

Experiments were carried out in two areas in Madagascar- in Ranomafana National park in 2007 and in Andasibe region in 2007 and 2008. In each area models were set in two experimental plots, namely in *Mantella* plot (plot where one of investigated *Mantella* species is found) and in control plot (area without any *Mantella* population). In Andasibe control plot was in Station forestière d'Analamazaotra (S 18°56.143' E048°24.879') and *Mantella* plot in Torotorofotsy (S 18°52.573' E048°22.243') populated with *M. aurantiaca*. In Ranomafana control plot consisted of several forest patches (near Val Bio biological station S 21°15.262' E047°25.302' and S 21°15.215' E047°25.288'; behind Ambatolahy village S 21°14.468' E047°25.591' and *Mantella* plot forest patches were found in Ranomafanakely (S 19°16.921' E041°18.818') and near Kidonavo bridge (S 21°13.522' E047°22.179'), populated by two *Mantella* species, *M. baroni* and *M. madagascariensis*. Control plots were chosen for the comparison of predator cognition of warning aposematic signals of *Mantella* frogs between the plots with "experienced" and "naive" predators. Additionally, data sets obtained in these experiments allowed me to test if there is the difference between predator learning effect between cryptic and aposematic non-edible prey. I compared the total amount of predation on each type of models. I presumed that predators learn quicker that some specific food (in this case clay models) is not edible when biting aposematic prey, and when biting cryptic prey the learning effect is presumed to take longer. When applied to

my experiment, we would expect that the rate of repeated predation on the same model will be higher on cryptic then on aposematic models.

4.2.3 Statistical analysis

Each model was assigned one value, which corresponds to the total amount of predation. This value was calculated as sum of predation for each model individually. Predations were considered 1) all together and then 2) separately diurnal and separately nocturnal predation at the beginning of the second season. Since the night period was longer than the day period, I standardized predation rates per hours. Diurnal predation was compared with nocturnal predation using Wilcoxon matched paired test. Data were analyzed both, considering all models sorted only based on colour morphology (shapeless models included in the analysis together with the frog models in matching colours), and with shapeless models excluded from the analysis. Distribution of total predation among three types of models was compared using chi-square tests, logistic regressions and Kruskal-Wallis ANOVAs separately for each plot. The same analysis was performed to test for differences between predation on cryptic (brown) versus aposematic models (black-yellow and orange taken together). Predation between shapeless and frog shape models, and predation between models set above the ground and set on the ground were compared with 2x2 chi square test.

Our assumptions for predator learning effects were tested with Kruskal-Wallis ANOVA. Statistical analyses were performed using StatSoft, Inc. (2005), STATISTICA (data analysis software system), version 7.1.

In order to test for effects of natural occurrence of *Mantella* species (comparison between plots with *Mantella* population and control plot) and colour of the model on the predation rate, data were analysed using generalised linear models (GLM) with binomial errors implemented in R 2.8.0 (R Development Core Team 2008) treating predation rate as dependent and plot and model colour as independent variables. Terms were deleted

sequentially from the full model using automated model simplification based on the Akaike Information Criterion (AIC, Burnham and Anderson 1998). The minimum adequate model was reached when no further deletions of points were possible without significant changes in deviance.

4.3 Results

4.3.1 Preliminary experiments in Braunschweig, Germany

In total 207 models were set in the field, 107 in the first and 100 models in the second series. Both, comparison between aposematic and cryptic models, as well as separate comparison between different colours showed no significant difference between predation on different colours of models when comparing only diurnal predation (2x2 chi square, $p=0.63$), but did show significant difference when comparing total predation (2x2 chi square, $p=0.02$). In both cases predation was higher on cryptic then on aposematic models.

4.3.2 Experiments in Madagascar

Total models distribution per site and plot is shown in Table 1.

Table 1. Number of models and predation records during experiments in Madagascar for each site and
plot. Plot abbreviations are as follows: AN *Mantella* = Andasibe *Mantella* plot, AN control= Andasibe control plot, Rf control= Ranomafana control plot, Rf *Mantella*= Ranomafana *M. baroni*/*M. madagascariensis* plot;, m.e. = missing data considered as void data, m.i.= missing data considered as predation.

Plot/season	Models set - including shapeless	Models set - without shapeless	Models predated - including shapeless; m.i.	Models predated - without shapeless; m.i.	Models predated - without shapeless, m.e.	Model/days - including shapeless	Model/days - without shapeless
AN *Mantella* 2007	-	466	-	250	197	-	1640
AN *Mantella* 2008	446	348	116	-	95	2231	1732
AN control 2007	-	448	-	235	206	-	1698
AN control 2008	460	364	94	-	76	2701	2082
Rf *Mantella*	352	276add	234	185	157	1775	1362
Rf control	334	264	214	166	139	1703	1309
Sum 2007	**1600**	**1454**	**933**	**836**	**699**	**6816**	**6009**
Sum 2008	**906**	**712**	**210**	-	**171**	**4932**	**3814**
Total	**2506**	**2166**	**1143**	**836**	**870**	**11748**	**9823**

During examination of the models in the field, each model was scored negative or positive for predation. In 2007, from 1600 models (1454 frog shape models), 933 (58.31%) models were predated when missing models count as predation (836 frog shape models; 57.50%), and 699 frog models (48.07%) when missing data considered as void data.

In the second season from 906 models altogether (of which 712 frog models) placed in the field in January-February 2008, 210 models, 23.18% (171 frog shape, 24.05%) were predated.

In two out of four plots in 2007 brown models sustained the highest predation compared to the two, conspicuous morphs (table 2; figure 2), although this difference was not significant (table 3). In 2008 in Andasibe *Mantella* plot, I found that orange models were more predated than the other two colours (though the difference was not significant), and in Andasibe control plot the most predated were again the brown models.

Table 2. Number of models and predation records for each colour separately during experiments in Madagascar, for each site and plot. For plot abbreviations see Table 1. Missing models are considered as void data.

Plot/ season	Brown models		*M. aurantiaca* models		*M. baroni/ madagascariensis*		Shapeless models	
	Total	Predated	Total	Predated	Total	Predated	Total	Predated
AN *Mantella* 2007	140	66 (47%)	163	66 (41%)	163	65 (40%)	-	-
AN *Mantella* 2008	113	28 (25%)	117	38 (33%)	118	29 (25%)	98	21 (21%)
AN control 2007	151	74 (49%)	149	72 (48%)	148	60 (41%)	-	-
AN control 2008	121	31 (26%)	122	22 (18%)	121	23 (19%)	96	18 (19%)
Rf *Mantella*	91	45 (49%)	93	55 (59%)	92	57 (62%)	76	42 (55%)
Rf control	83	46 (55%)	90	42 (47%)	91	51 (56%)	70	36 (51%)

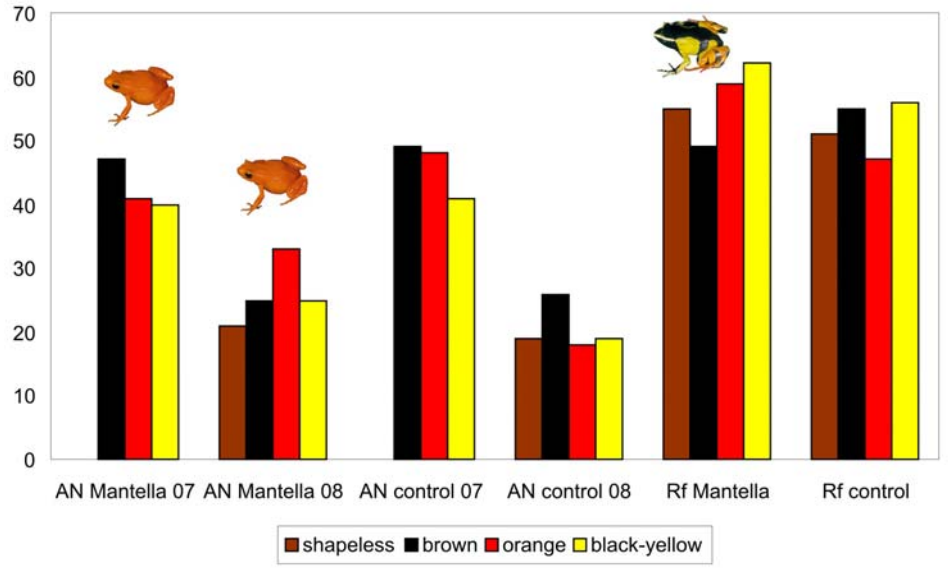

Figure 2. Distribution of predation in all plots shown in percentage of total number of models set in each colour. For plot abbreviations see Table 1. Missing models are considered as void data.

I performed a generalised linear model with predation rate as dependent, and plot and model colour as independent variables. After stepwise deletion of non significant terms, the model revealed that in Andasibe 2007 (d.f.= 913) and Ranomafana 2007 (d.f.= 539), neither the natural occurrence of *Mantella* nor the colouration of the model caused differences in the

predation rate, as all terms could be removed from the model. In Andasibe 2008 the apparently higher predation on orange models in *Mantella* plot compared to the control plot received statistical support because interaction term of plot and orange colouration was significant (d.f.= 706, p=0.04). There was no further difference in the predation rate between plots or different colour of the models (d.f.= 706, p>0.15).

Table 3. Summary of analyses of total predation compared to colour of models. Method abbreviations are as follows: K-W ANOVA= Kruskal-Wallis ANOVA, Log.Reg= Logistical regression, Chi ^{2}= Chi square, No SL= shapeless models excluded from the analysis, m.e. = missing models considered as void data. For plot abbreviations see Table 1

Method	**K-W ANOVA**		**Log. Reg.**		**Chi ^{2}**	
Plot	SL	No SL/ m.e.	SL	No SL/ m.e.	SL	No SL/ m.e.
AN *Mantella* 2007	-	p=0.001/ p=0.150	-	p=0.012/ p=0.454	-	p=0.126/ p=0.569
AN *Mantella* 2008	p=0.368	p=0.278	p=0.401	p=0.304	p=0.509	p=0.420
AN control 2007	-	p=0.056/ p=0.122	-	p=0.242/ p=0.266	-	p=0.509/ p=0.489
AN control 2008	p=0.264	p=0.308	p=0.225	p=0.244	p=0.332	p=0.372
Rf *Mantella*	p=0.194	p=0.220/ p=0.408	p=0.257	p=0.214/ p=0.201	p=0.636	p=0.601/ p=0.501
Rf control	p=0.129	p=0.156/ p=0.717	p=0.358	p=0.502/ p=0.929	p=0.657	p=0.752/ p=0.628

Analyses comparing predation between cryptic and aposematic models showed similar results; no significant difference between the predation rate on either of the groups was found (table 4).

Table 4. Summary of analyses of total predation compared to colour type of models (aposematic and cryptic). For plot abbreviations see Table 1; for method abbreviations see Table 3.

Method	**Kolmogorov-Smirnov**		**Log. Reg.**		**Chi** ^{2}	
Plot	SL	No SL/ m.e.	SL	No SL/ m.e.	SL	No SL/ m.e.
AN *Mantella* 2007	-	p< 0.050/ p > 0.100	-	p=0.005/ p=0.211	-	p=0.005/ p=0.163
AN *Mantella* 2008	p > 0.100	p > 0.100	p=0.529	p=0.464	p=0.529	p=0.464
AN control 2007	-	p > 0.100/ p > 0.100	-	p=0.119/ p=0.360	-	p=0.119/ p=0.360
AN control 2008	p > 0.100	p > 0.100	p=0.085	p=0.099	p=0.084	p=0.099
Rf *Mantella*	p > 0.100	p > 0.100/ p > 0.100	p=0.937	p=0.587/ p=0.080	p=0.636	p=0.601/ p=0.501
Rf control	p > 0.100	p > 0.100/ p > 0.100	p=0.438	p=0.758/ p=0.912	p=0.937	p=0.587/ p=0.083

Comparison between diurnal and nocturnal predation showed no significant difference either, although the predation was slightly higher during the night. The results are shown in Tables 5-6.

Table 5. Separated diurnal and nocturnal predation on each colour of models, shown as absolute value and as percent of total numbers of models set in each colour. For plot abbreviations see Table 1.

	AN *Mantella*	AN *Mantella*	AN control	AN control
Colour	**Day**	**Night**	**Day**	**Night**
brown	6 (11.8%)	9 (17.7%)	7 (13.0%)	6 (11.1%)
orange	7 (13.2%)	6 (11.3%)	0 (0%)	3 (6.3%)
black-yellow	5 (9.3%)	10 (18.5%)	1 (1.8%)	6 (10.7%)

Table 6. Comparison of diurnal and nocturnal predation in 2008; Wilcoxon matched pair test (shapeless models excluded from the analysis). day vs night= total diurnal compared to total nocturnal predation; day/h vs night/h= standardized diurnal compared to standardized nocturnal predation. For plot abbreviations see Table 1.

Plot		p
AN *Mantella*	day vs night	0.288
AN *Mantella*	day/h vs night/h	0.109
AN control	day vs night	0.263
AN control	day/h vs night/h	0.429

Analysis between predation on frog shaped models and shapeless models showed no significant difference (table 7), although the predation was higher on frog shaped models.

Table 7. Comparison of predation on shapeless and frog shaped models; Log. Reg= Logistic regression, Chi ^{2}= 2x2 chi square test. For plot abbreviations see Table 1.

Site	**Log. Reg.**	**Chi ^{2} m.i./ m.e.**
AN *Mantella* 2007	-	-
AN *Mantella* 2008	p=0.242	p=0.242
AN control 2007	-	-
AN control 2008	p=0.679	p=0.645
Rf *Mantella*	p=0.000	p=0.347/ p=0.845
Rf control	p=0.030	p=0.647/ p=0.602

Testing of predator learning effects showed that there was a significant difference between single and repeated predation in all plots in 2007 (tables 8-9); brown models sustaining higher multiple predation in all plots when including missing models as predated, and in two plots when missing models are treated as missing data. These results were not confirmed by data from 2008.

Table 8. Absolute numbers of single (sg) and repeated (rep) predation in each plot separately for each colour of models. For plot abbreviations see Table 1.

	AN *Mantella* 2007	AN *Mantella* 2008	AN control 2007	AN control 2008	Rf *Mantella*	Rf control
Colour	sg/ rep	sg/ rep	sg/ rep	sg/ rep	sg/ rep	sg/ rep
brown	41/ 24	25/ 3	47/ 27	27/ 4	27/ 18	25/ 21
orange	51/ 15	31/ 7	56/ 16	17/ 5	35/ 20	33/ 15
black-yellow	55/ 10	24/ 5	48/ 12	21/ 2	38/ 19	37/ 14

Table 9. Comparison of single and repeated predation; Kruskal-Wallis ANOVA. For plot abbreviations see Table 1.

Plot	**With sl**	**No sl /m.e.**
AN *Mantella* 2007	-	p=0.019/ p=0.009
AN *Mantella* 2008	p=0.729	p=0.678
AN control 2007	-	p=0.031/ p=0.042
AN control 2008	p=0.272	p=0.366
Rf *Mantella*	p=0.144	p=0.026/ p=0.716
Rf control	p=0.202	p=0.045/ p=0.141

Comparison on the predation between models set on the ground and above the ground seem to be significant in some of the plots (table 10-11).

Table 10. Absolute numbers and percentage of total number of models set in each colour on or above the ground, for each plot separately. For plot abbreviations see Table 1.

	AN *Mantella* 2007	AN *Mantella* 2008	AN control 2007	AN control 2008	Rf *Mantella*	Rf control
ground	151 (48.4%)	40 (22.2%)	121 (50%)	45 (19.1%)	80 (62.5%)	106 (65.0%)
above	99 (64.3%)	55 (32.7%)	121 (59.6%)	31 (24.0%)	102 (72.3%)	57 (59.4%)

Table 11. Comparison of predation rate on models set on the ground and above the ground; 2x2 chi square test. For plot abbreviations see Table 1.

Plot	**With sl**	**No sl/ no mising**
AN *Mantella* 2007	-	p=0.001/ p=0.008
AN *Mantella* 2008	p=0.004	p=0.028
AN control 2007	-	p=0.090/ p=0.051
AN control 2008	p=0.267	p=0.273
Rf *Mantella*	p=0.049	p=0.071/ p=0.887
Rf control	p=0.401	p=0.363/ p=0.437

4.3.3 Predator identification

A very important issue in these experiments is to identify which predator has caused the observed bite marks in the models. This was possible only in several cases. Figure 3 shows proportion of predator categories as inferred from the type of bite marks for each of studied plots and seasons.

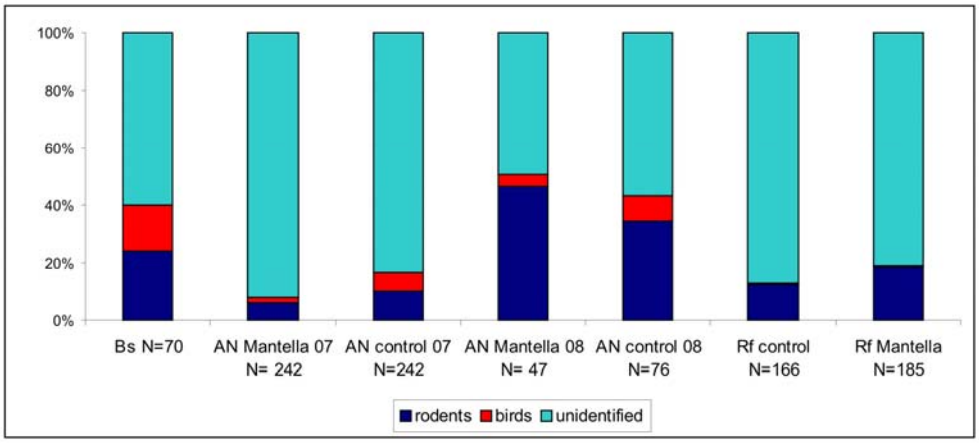

Figure 3. In 2007 in Andasibe *Mantella* plot 15 bite marks recorded were made by rodents, 4 by birds; in Andasibe control plot in 2007, 25 biting marks recorded were by rodents, 16 by birds, in Ranomafana *Mantella* plots 34 biting marks recorded were made by rodents, 1 by bird, in Ranomafana control plot 21 bite marks recorded were made by rodents, 1 by bird. In Andasibe *Mantella* plot in 2008, 44 bite marks recorded were made by rodents, 4 by birds; in Andasibe control plot in 2008, 26 bite marks recorded were by rodents, 7 by birds. In Braunschweig 17 biting marks recorded were made by rodents, 14 by birds. Bs= Braunschweig; for other plot abbreviations see Table 1.

Predation by birds in Andasibe control plot in 2007 and predation by birds from Braunschweig were reanalysed separately. In Andasibe control plot in 2007 I found no significant difference between predation on different colours of models (chi square, p= 0.395), similar like there is no significant difference when comparing predation and type of colouration (aposematic-cryptic, 2x2 chi square, p= 0.497) and comparison between single and multiple predation (K-W ANOVA, p= 0.291). On the other hand there is a significant difference between predation on brown (p= 0.025) and black-yellow models (p= 0.049) when set on the ground and above the ground; brown models being predated more often by birds when set on the ground, and black-yellow and orange models being predated more often when set above the ground (although this difference for orange models was not significant, p= 0.450).

In Braunschweig, I found no significant difference between predation on different colours of models (chi square, p= 0.170), but when comparing predation on cryptic and aposematic models, there was a significantly higher predation on cryptic models (2x2 chi square, p= 0.050).

4.4 Discussion

In this study of the efficiency of aposematic colouration in *Mantella* frogs, my predictions of predators' behaviour based on their presumed previous experience were not confirmed. In *Mantella* plots predators were not particularly deterred by those models that resembled the aposematic frogs inhabiting the plots. In most of the studied plots, brown models sustained the highest rates of predation. This could be partially interpreted by the reluctance of predators for eating novel prey, i.e. neophobia (Lindström *et al*. 2001). If the avoidance of brightly coloured models would be stronger in *Mantella* plots then in control plots, this could be a result from predator learning coupled with unspecific recognition (Pavlov 1927). My results do not support this hypothesis, and avoidance of predators towards bright models was found more often in almost all experimental plots, independent from whether the respective aposematic colour was present or not in the corresponding plot, except in Andasibe 2008. In this case the comparison between *Mantella* and control plot showed different results from the one I expected. My hypothesis that predators in *Mantella* plot would avoid the aposematic colour they had the opportunity to learn, more than the other they never encountered, was thus not confirmed by my data. On the contrary, in Andasibe *Mantella* plot in 2008 orange models resembling *Mantella aurantiaca* (the *Mantella* species inhabiting the area) was the most commonly predated.

Despite the fact that in the final analysis I have not separated diurnal from nocturnal predation, a preference for one type of models based on olfactory cues (in nocturnal predators, i.e. mammals) is unlikely. Preliminary test performed to check this assumption, using pieces of clay that were given to the laboratory mice and checked for bite marks at the end of the day period as well as at the end of the night period, showed no significant difference. As well, the effort to separate diurnal from nocturnal predation may not be very successful. Two important periods are for practical reasons excluded from diurnal predation, although they do belong to it- dusk and dawn. During this time, it is too dark to check the models for

predation marks in the field, but on the other hand, it is still bright enough that predators could recognize the colours. Additionally, these periods are also the time of the day when many of the predators have their activity peak. Also checking of models two times a day leaves very short time period for predators to attack, since the person checking the models is present at the plot for several hours per day. Based on these arguments, I think that comparison of diurnal and nocturnal predation was not very reliable.

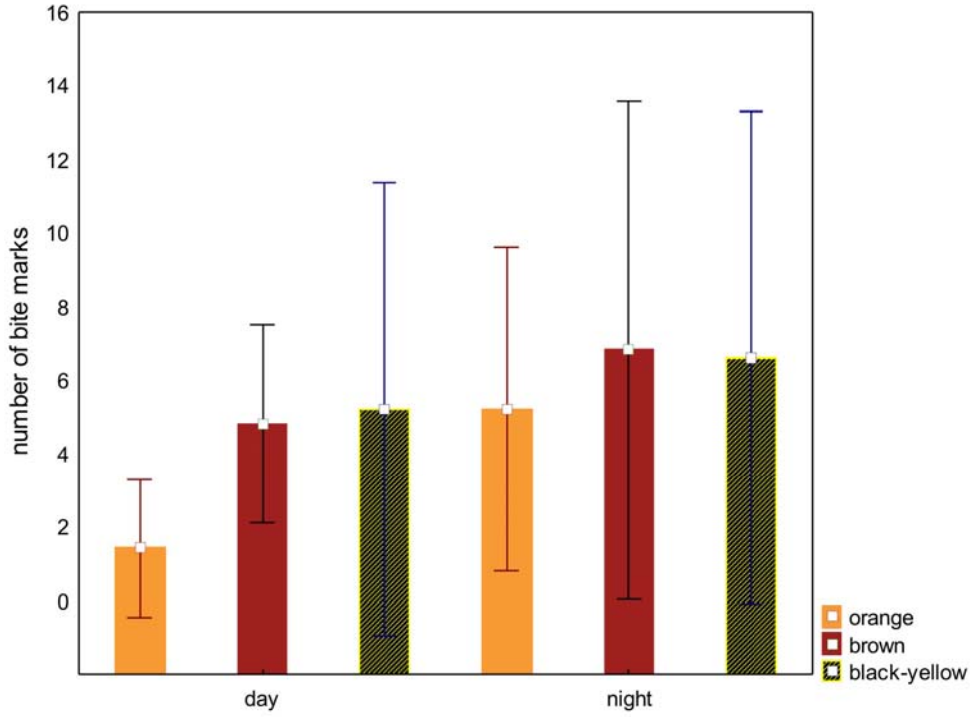

Figure 4. During mice clay experiments, total rate of predation for all mice together was higher during the night then during the day. Statistical analyses showed no significant difference for both diurnal (p=0.33) and nocturnal predation (p=0.82) between different colours.

The fact that there was no significant difference on predation between frog and shapeless models can imply that some of the models were possibly not seen as frogs, but as some fruits or seeds, indicating that this type of experiment may not be suitable for this type of research in Madagascar.

In general, I can say that models set above the ground are equally likely to be predated as those set directly on the ground (table 8). Although the different predation rate seems to be determined by the position of models when observing the statistical results separately, the comparison on the

predominated position varies between sites, plots and seasons (e.g. in Andasibe *Mantella* plot in 2007 the higher predation was observed on models set on the ground, while in 2008 the models set above the ground sustained significantly higher predation). On the contrary, comparison of bird predation in Andasibe control plot in 2007 showed a significant difference between predation rates depending on the position of the model. Brown models were significantly more predated when set on the ground. On the other hand, conspicuous models (both black-yellow and orange, although orange not significantly) sustained higher predation when set above the ground.

In Braunschweig, evaluation of bird predation marks showed a significant difference between predation depending on the type of the model (cryptic, aposematic), with cryptic models being more predated. This can be explained similarly like in Madagascar, by neophobic predator reactions towards new conspicuous prey (Lindström *et al*. 2001).

First frog model experiments were carried out in Costa Rica in 2005 by Anderson (2006) who was examining the aposematic colouration of poisonous dendrobatid frog *Oophaga pumilio*. He used two different types of models- red with blue legs and uniformly coloured brown models. His results showed no preference towards any type of models. On the contrary, Saporito *et al* (2007b) who used the same colour combinations of models and repeated the experiments in the same area, found significantly higher predation on brown models then on the red-blue models. Great difference between the two experiments was the sample size; Saporito *et al* (2007b) increased the sample size for more then two times. From total 800 models they set in the field, 12.4% of the models were predated and 3 % missing. Additionally, most of the predations recorded were by birds (72%) who are visually oriented predators, implying that the colouration of *O. pumilio* is aposematic (at least towards bird predators). Not so clear and unambiguous results were presented by Noonan and Comeault (2009). They set three different types of models in the field- brown models, yellow-black models resembling local form of *Dendrobates tinctorius* and black-blue with yellow

stripe resembling novel frog form for this area. They recorded in total 11% of the models as predated, and 21% of the predation was assigned to birds. Predation by other predators showed no significant difference according to the colour of the models. On the other hand, birds showed significant preference towards the new aposematic model, over the other two types probably indicating the avoidance of local aposematic form. Both experiments from Saporito *et al*. (2007b) and Noonan and Comeault (2009) show results that differ from ours, confirming the hypothesis of the aposematic colouration of these two frogs.

Predation on members of the genus *Mantella* is probably rare, due to the presence of toxic skin alkaloids, which they share with dart-poison frogs (Dendrobatidae). Up to date there are only few predation records on *Mantella* frogs. Two of them were observed on ***M. laevigata*** in Nosy Mangabe in Northeastern Madagascar by Heying (2001). She reported one successful predation by *Zonosaurus madagascariensis*, a common gerrhosaurid lizard, and aborted predation attempt by *Boa madagascariensis*. Additionaly, I report here on two successful predations on ***M. aurantiaca*** in Torotorofotsy wetland in central east Madagascar. First predation was most probably by the same lizard species, *Zonosaurus madagascariensis* and the second predation was by a snake from genus *Thamnosophis*, most likely ***T. lateralis*** (Chapter 6).

Several other experiments with clay models have been performed, but until now, these experiments are the first of that kind in Madagascar. So far, frog clay models experiments performed by other researchers mostly supported the hypothesis of aposematism, but ours did not. There are several possible reasons for that. One of the reasons is that the composition of predators in Madagascar differs from predators found in other tropical countries, namely bird fauna. Study of the bird fauna and predation on amphibians carried out in central Panama (Poulin *et al*. 2001) showed that 16 species of insectivorous birds predate on amphibians. Such a predation is considered to be opportunistic, and depends on the frog availability. Time

period when frogs are the most abundant corresponds to lowest availability of arthropod prey.

In Madagascar bird fauna is characterized by is its relative poverty in number of species, not only in comparison with the continental bird communities at the same latitude but also in comparison with the bird population of such Indo- Oceanic islands as Borneo. It has very high level of endemism, in terms of both genera (around 25%) and species (more then 50%) and almost all the endemic avifauna consists of forest species (Langrand 1990).

For this reason, many niches usually filled by birds are in this case occupied by other animals like for example *Daubentonia madagascariensis*, a lemur which is thought to fill the niche of the woodpecker (Cartmill 1974). This can significantly influence the results of my experiments, and comparing the percentage of bird and mammal predators, we can see that bird predation can almost be neglected. Most of unidentified predations probably belong to rodents or insectivores like tenrecs, and some to reptiles, even if many of the reptiles would presumably not bite a still prey.

Anurans are known to be preyed upon by many predators that it has been stated by Duellman and Trueb (1994) that 'practically anything will eat an amphibian'. This statement was well supported in the study carried out by Toledo *et al*. (2007) who emphasize the diversity of anuran vertebrate predators. They reported anurans to be preyed upon even if they had a defensive mechanism (large amounts of skin toxins) found for example in bufonids (e.g. *Bufo proboscideus*, Menin 2005; *Leptodactylus pentadactylus* Roberts 1997) and denrobatids (e.g. *Dendrobates auratus*, Hedstrom and Bolanos 1986; Master 1998; Gray *et al*. 2002; *Oophaga pumilio*, Daly, pers. comm.; Donnelly, pers. comm.). Based on numerous unpublished data, articles and natural history notes published in *Herpetological review*, they found snakes to be the main anuran predators, suggesting that they drive the diversification of anuran defensive strategies.

5 Unpalatability of *Mantella* and snake feeding choice experiments

Abstract

Aposematism is an association in a prey organism of warning signals and unprofitability to predators. One example of aposematism are Madagascan poisonous frogs from the genus *Mantella*. I tested their efficiency in predator deterring based on visual and olfactory cues, by feeding choice experiments that were carried out in Madagascar in 2007 and 2008, with several species of snakes that were caught in the field. Snakes were offered one *Mantella* and one edible, non-alkaloid containing, non-conspicuous frog at the same time, giving them the opportunity to choose. They showed a strong preference for edible, non-alkaloid containing, non-conspicuous frogs over specimens of *Mantella*, both in 2007 and 2008. In 2008 I also compared snake predation rate on two types of frogs between "experienced" snakes (caught in *Mantella* habitat) and naïve snakes (caught in areas not inhabited with *Mantella*) and found significant differences, with "experienced" snakes eating altogether only one *Mantella* frog.

Keywords: prey-choice experiment, Madagascar, Amphibia, *Mantella*, Reptilia, Serpentes, aposematism

5.1 Introduction

Aposematism, a phenomenon of defended prey organism that advertises its noxiousness, is based on predators' ability to learn more readily to recognize unpalatable prey when being conspicuous (Cott 1940; Edmunds 1974; Guilford 1990). We know little about how predators process visual and chemosensory cues about aposematic prey, as well as it is not clear how this interacts with recognition and avoidance of such prey (Guilford 1992; Terrick *et al*. 1995). In general conspicuousness of a prey is a reliable indicator of prey unpalatability and/or noxiousness.

Anuran amphibians are generally seen as cryptic, although few groups are considered to be aposematic (Santos *et al*. 2003; Vences *et al*. 2003; Darst *et al*. 2006). Colouration in anurans was recently studied by Toledo and Haddad (2009) who divided it into three major categories: mimicry, deceptive colouration and aposematism; the latter involving unpleasantness or danger, and generally being contrasted to the background. Apart from pigments and numerous biologically active compounds (e.g. peptides) a few groups of frogs contain alkaloids in their skin that they accumulate and sequester from the diet into their skin glands (Daly and Myers 1967; Daly *et al*. 1999; Saporito *et al*. 2007a).

Predation on alkaloid containing poisonous frogs is thought to be rare, and there are only about ten predation reports on dendrobatid frogs (e.g. Daly and Myers 1967; Brodie and Tumbarello 1978; Myers *et al*. 1978; Fritz *et al*. 1981), and only four on *Mantella* frogs (Heying 2001; this dissertation Chapter 6). Snakes are the most common amphibian predators (Toledo *et al.* 2007) but despite their importance as amphibian predators, only several snake feeding experiments with amphibian prey were performed (e.g. Mori 1989; Williams *et al.* 2003). On the other hand, there are a few feeding experiments with naïve avian predators to assess their learning abilities that used dendrobatid frogs as prey (Darst and Cummings 2006; Darst *et al*. 2006). In this context, feeding experiments with *Mantella* are now reported for the first time.

Overall high biodiversity found in Madagascar refers also to Malagasy reptiles; from 370 described species, 92% are endemic. Most of Malagasy snakes belong to family Colubridae that contains 18 genera and 75 species in Madagascar (Glaw and Vences 2007), being the biggest snake family in general. All snake species used in my study belong to this family as well.

In this study I tested the influence of snakes' previous experience, and their learning abilities in correlation to predation on aposematic coloured amphibian prey.

5.2 Materials and methods

Snakes that were used in my experiments were all caught in the wild, and after the experiments were finished, released back to the same place they were caught. Specimens that could not be released to the site of the origin were euthanized by injecting chloro-butanol and catalogued as a part of the zoological collection of Miguel Vences. Part of the snakes were caught in areas where no *Mantella* populations are found (naïve snakes), while some snakes caught, live in syntopy with one of *Mantella* species (*M. aurantiaca*, *M. crocea*; "experienced" snakes). Each snake was kept separately in an improvised non transparent cage. Water was provided *ab libitum*. Methodological approaches applied in the two seasons differed slightly between each other. In 2007 frogs were given to snakes and observed until they were eaten/ the experiment was over, and in 2008 frogs were offered on each occasion for a period of 90 minutes.

5.2.1 Snake feeding experiments in 2007

Six snakes (three *Compsophis laphystius*, two *Liopholidophis sexlineatus* and one *Thamnosophis lateralis*) were caught in the wild in Ranomafana region. Each snake was offered two frogs at the same time - one edible frog (mostly *Guibemantis liber*), and one noxious *Mantella baroni*, so the snake had the opportunity to choose which frog to eat. The term "edible frog" refers to any non-alkaloid containing and non-conspicuous frog. After 24

hours I checked the results: did the snake eat any of the two frogs offered, and if it did, which frogs were eaten? A new trial with the same snake was performed several days after the last ingestion of a frog. One specimen of *C. laphystius* was used as a control, being offered only edible frogs. Altogether 137 frog/days were offered to the snakes, of which 68 *M. baroni* and 69 non-conspicuous frogs. Experiments were performed between January and March 2007, in Ranomafana National Park, in Madagascar.

5.2.2 Snake feeding experiments in 2008

Altogether 16 snakes were caught from different areas in Madagascar; seven near Andasibe village (S 18°56.143' E048°24.879'; naïve snakes; one *Ithycyphus perineti*, two *Liopholidophis sexlineatus,* one *Thamnosophis epistibes* and two *T. infrasignatus*), three in Mantadia ("experienced" snakes; *T. epistibes*, *T. infrasignatus* and *T. lateralis*), near the *Prolemur* research camp (S 18°46.114' E048°25.389') where a *Mantella crocea* population is found, one in the Torotorofotsy wetland (S 18°52.573' E048°22.243'; "experienced" snake; *T. infrasignatus*) where a *M. aurantiaca* population is found, and six in Ranomafana National Park in areas without *Mantella* populations (naïve; four *Compsophis laphystius*, one *C. boulengeri* and one *L. rhadinaea*).

During the experiments, each snake was placed in a white transparent plastic box (arena) with closable lid, for 90 minutes. It got two live frogs offered, one individual of *Mantella aurantiaca* and one of an edible frog (*Guibemantis liber, Mantidactylus betsileanus, Gephyromantis boulengeri, Boophis rappioides* or *Ptychadena mascareniensis*). During the first two trials, frogs were allowed to move freely in the arena, and this seems to have confused the snakes. By jumping around, frogs would leave their chemical cues everywhere, and the snake was not able to distinguish which odour belongs to which frog, and as a consequence the snakes did not attack any of the frogs offered. In order to avoid this problem, the frogs' movements were limited to ca 20 centimetres in all directions, by putting frogs on a leash,

under their forelimbs and fixing it to the floor of arena. After 90 minutes, I recorded if any of the frogs was eaten, and which. Following the experiment, the snake was returned to its cage. After every trial, the plastic box was thoroughly washed and dried. In some cases, experiments were filmed, and time to first attack on each frog, as well as time when each frog was eaten, were recorded. Each snake was used in several trials of the same experiment, and the number of trials was variable between snakes, which was dependent on the time when each individual snake was caught. Snakes caught during the first weeks of the experiment were used in more trials then those caught later.

Additionally, I used some snakes as a negative control, by offering only one edible frog at the time during 90 minutes and the snake reaction was recorded. Altogether 108 frogs were offered: 50 *Mantella* and 58 edible frogs (eight of which were used as control). The experiments were carried out between January and March 2008, in Madagascar, in the village of Andasibe.

5.2.3 Unpalatability and brightness of Mantella crocea and M. aurantiaca

In Mantadia, while searching for snakes for feeding experiments, we also caught two specimens from the same population of different colour morphs of *M. crocea* (orange-reddish and yellowish), and took photos of the frogs' dorsal and ventral view. For comparison, colouration of two *M. aurantiaca* specimens was analysed. A Kodak colour separation guide was added to each image to enable colour corrections. Photos were taken with compact digital camera Pentax Optio W20, in diffusely lighted photo-box under standardized light conditions. Light sources comprised two Kaiser 5454 daylight lamps producing colour temperature of 5400K. Colouration of the frogs was analyzed using Adobe Photoshop. Prior to the analysis, photos were calibrated by adjusting the values for white and black colour on Kodak colour separation guide (255 and zero respectively). In order to calculate mean brightness of dorsal and ventral pattern, the surface of the frog was

equally divided into six fields. Values for each field were written down and the mean value for each frog was calculated. Brightness was calculated via HSP colour model (brightness= sqrt (0.241 R^2 + 0.691 G^2 + 0.068 B^2)) (http://alienryderflex.com/hsp.html HSP Color Model — Alternative to HSV (HSB) and HSL *©2006 Darel Rex Finley (last accessed on 30. June 2009)*).

Apart from brightness analysis, I compared the unpalatability of these two *Mantella* populations in two-bottle experiment with domestic mice (*Mus musculus*). I used 20 mice (ten males and ten females) divided into four groups of five. Each group had food provided *ab libitum*. In 24 hour intervals, each group was offered two bottles - one containing 1% ethanol solution (control), and another containing diluted *Mantella* skin extract disolved in 1% ethanol solution (since frog alkaloids are water insoluble). The amount drank from each bottle was noted. Experiments were performed at the end of January beginning of February 2008, at the University of Antananarivo, in Madagascar. This method had previously been tested in Braunschweig with sparteine solution (a commercially available alkaloid) and *Mantella* skin samples that were previously available.

5.2.4 Statistical analysis

Comparison between predation rates on *Mantella* frogs and edible frogs was tested with chi square test (2x2 test). The same test was used to compare the predation rate between naïve snakes that were caught in non-*Mantella* sites, and "experienced" snakes caught in *Mantella* sites. In the latter case, I tested separately predation rate on *Mantella* frogs and separately on edible frogs. Since some of the snakes did not eat any of the frogs during all the trials, I omitted them from the analyses. The learning effect (based on predation rate on *Mantella* frogs) between different trials was tested with repeated measures ANOVA. In the last five trials only four or fewer snakes were used. This presented too small sample size for statistical analysis, so only first four trials were included in this analysis. Due to a small number of

snakes from the same species, all the snakes were pooled together and analysed as equivalent. Similar was applied to the analysis between naïve and "experienced" snakes where all the specimens caught in a *Mantella* site were analysed together, as well as all specimens caught both in Andasibe and Ranomafana. Unpalatability of alkaloid solution compared to control solution was examined with Wilcoxon matched paired test, and differences of unpalatability of alkaloid samples were assessed with Kruskal- Wallis ANOVA. Prior to analysis, data were standardized for each mouse separately. All statistical analyses were performed using sing STATISTICA 7.1. (data analysis software system; StatSoft, Inc. 2005).

5.3 Results

5.3.1 Snake feeding experiments in 2007

In the four weeks of this experiment, 43 frogs were eaten by snakes; nine individuals of *M. baroni* and 34 individuals of edible frogs. The control snake ate five frogs. Results for each individual snake are shown in figure 1 and in Appendix, table 2. Statistical analysis of predation on *Mantella* and edible frogs showed significant difference between predation rate on these two types of prey (χ^2=12.24, *d.f.*=1, p=0.001).

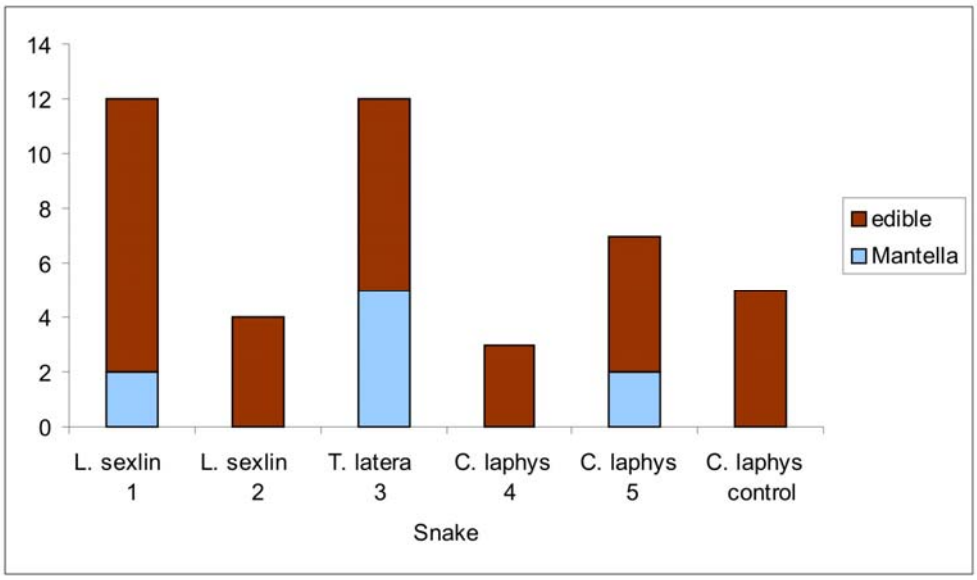

Figure 1. Number of frogs eaten during the experiments in 2007 (y axis) by six snake specimens. L. sexlin= *Liopholidophis sexlineatus*, T. latera= *Thamnosophis lateralis*, C. laphys= *Compsophis laphystius*. The last snake C. laphys was a control snake, being offered only edible (brown) frogs.

5.3.2 Snake feeding experiments in 2008

Out of 16 snakes that were used during the experiments, only ten of them ingested at least one of the frogs offered. The other six snakes (two specimens of *L. sexlineatus* and *C. boulengeri* and one specimen of *T. infrasignatus* and *C. laphystius*) that did not ingest any of the frogs were excluded from the analysis. I analyzed total predation on *Mantella* and edible frogs among all snakes together, and the results show a significant difference between predation rate on these two types of prey (χ^2=4.49, *d.f.*=1, p=0.034; figure 2 and Appendix, table 3). Total predation rate on *Mantella* frogs was compared between naïve and "experienced" snakes and showed a significant difference (χ^2=6.730, *d.f.*=1, p=0.010) between the two snake groups (figure 3). On the other hand, predation rate on edible frogs seems to be correspondent among both naïve and "experienced" snakes (χ^2=0.23, *d.f.*=1, p=0.629; figure 3).

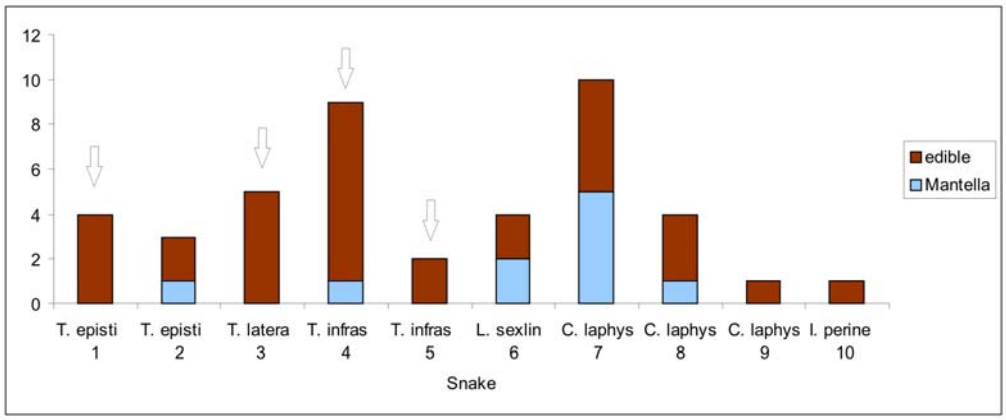

Figure 2. Number of frogs eaten during the experiments in 2008 (y axis) by ten snake specimens. T. episti= *Thamnosophis epistibes*, T. infras= *T. infrasignatus*, I. perine= *Ithycyphus perineti;* for other abbreviations see Figure 1. Arrows indicate "experienced" snakes.

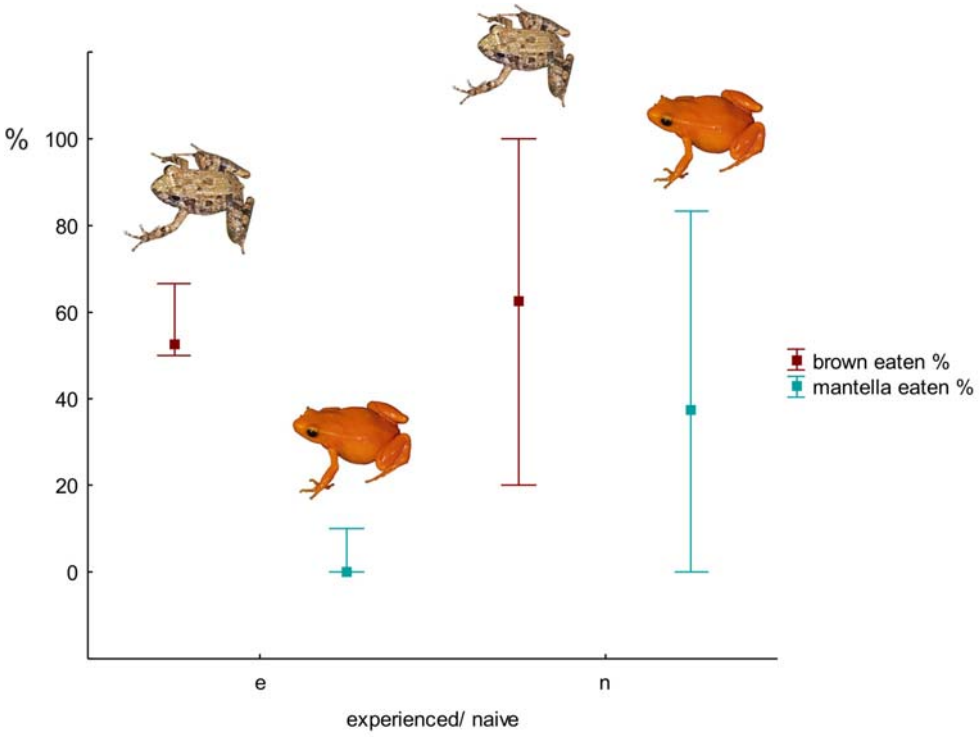

Figure 3. Percentage of *Mantella* and edible frogs eaten by "experienced" snakes (left two bars) and naïve snakes (right two bars).

Repeated measures ANOVA analysis of the first four trials for all snakes showed no significant differences in predation rate between the two types of frogs (p=0.488). When plotted on the graph (figure 4), we can clearly see that edible frogs were in general eaten more often then *Mantella* frogs

(except in trial 3 when the number of both types of prey eaten was equal), but because of a small sample size, this difference was not statistically significant. In the last four trials we can see that none of *Mantella* frog was eaten, but due to the low number of snakes used in these trials, I could not test it.

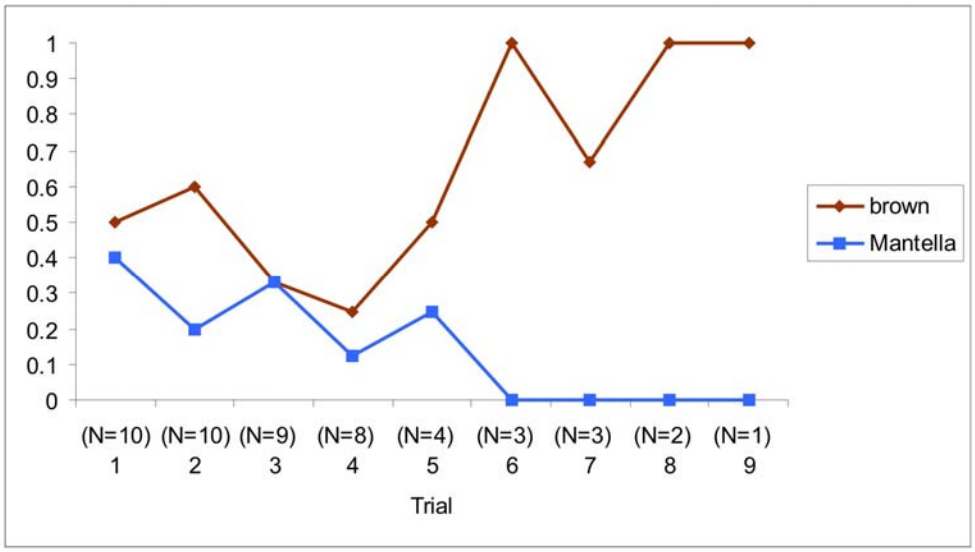

Figure 4. Percentage of frogs eaten in each separate trial during the experiments in 2008 (y axis). Number of edible and *Mantella* frogs offered was the same in every trial. (N) represents the number of each type of prey offered to the snakes in every consecutive trial.

In control experiments, when only one edible frog was offered, only in two cases the snake did not eat the frog.

5.3.3 Unpalatability and brightness of Mantella crocea and M. aurantiaca

The dorsal colour pattern of both *Mantella aurantiaca* specimens examined is brighter (brightness=116 and 87) than in *M. crocea* (brightness 77 and 66), and in the ventral colour pattern, the situation is exactly the opposite (160 and 173 for *M. crocea* and, 101 and 114 *M. aurantiaca*).

In two-bottle mice experiments, I confirmed that generally the volume of control solution drank was higher than the volume of alkaloid solution ($Z=2.55$, $p=0.011$; figure 5). I also showed that there is a significant difference between the volumes drank of different alkaloids samples, suggesting that alkaloids from some individuals may be more unpalatable

than others (H=57.724, p=0.000; *M. aurantiaca* skin extract seemed to be the least unpalatable).

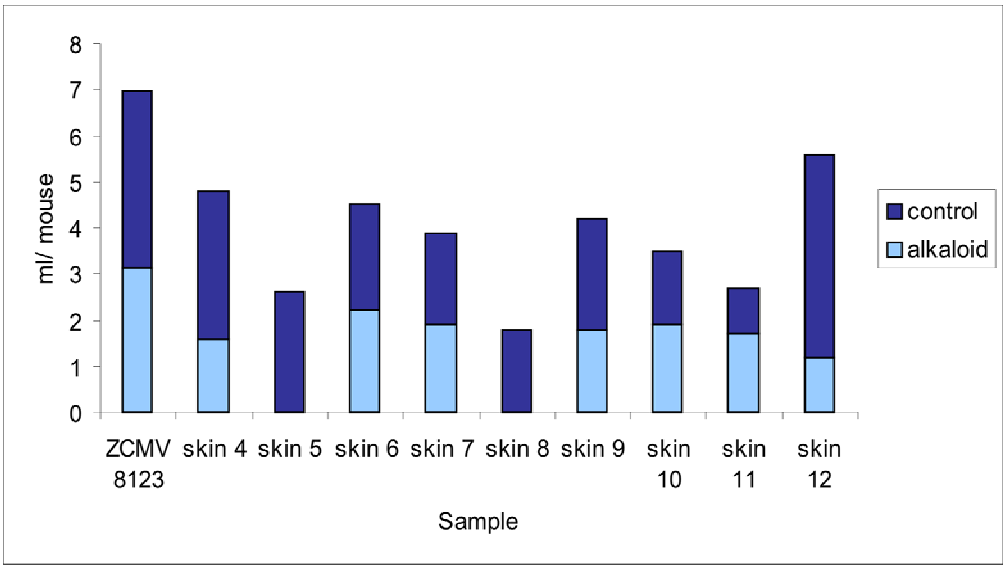

Figure 5. Volume (mean values) of alkaloid and control solutions drank per mouse, in two-bottle experiments. ZCMV 8123- *M. aurantiaca* skin extract, skin 4- 12 *M. crocea* and *M. baroni* skin extracts.

5.4 Discussion

In both experimental seasons, I confirmed that there is a significant difference between predation on *Mantella* frogs and edible frogs, implying that the snakes can distinguish poisonous frogs from edible once. In 2008, we could see that in the first trials, predation on *Mantella* was higher then in the last trials, indicating that snakes are able to learn to distinguish edible and unapalatable prey. Possibility of existing interspecific and preferences on population level for a certain prey type can be excluded (e.g. one specimen of *C. laphystius* ate five *Mantella* frogs, while other two snakes ate one and none *Mantella*, respectively; all specimens came from Ranomafana). A learning effect was shown already in the first trial by comparison of naïve and experienced predators, with the latter eating in total only one *Mantella* frog, in the first trial. Experienced predators were apparently familiar with the unpalatability of *Mantella* frogs and were able to distinguish different prey types based on chemosensory and olfactory cues. The recognition was not based on colouration and this was evident

from avoidance of experienced predators against the new species of *Mantella* which they had not encountered in their natural habitat and which had different colour patterns (except for one snake, from Torotorofotsy; three of the snakes should have been familiar with *M. crocea* which is yellowish with dark lateral pattern and not bright orange as *M. aurantiaca*). In both seasons, I observed one snake individual eating all the frogs offered; in 2007 a specimen of *T. lateralis* and in 2008 *C. laphystius*. From my experiments we can conclude that *Mantella* toxins are not deadly for snakes, since none of the snakes that ate *Mantella* frog died.

The difference found between experienced and naïve snakes is consistent with the finding of Burghardt (1966; 1969; 1970; Burghardt and Abeshahen 1971) showed that there can be an innate aversion towards different prey (on the example of genus *Thamnophis*). This behavioural variation found in new born snakes can be of genetic origin, hunger level and experience. It was also proved that snakes learn faster to avoid aposematic then cryptic prey. In the absence of additional stimuli reinforcement, learned aversion in garter snake does not persist indefinitely. As proposed by Terrick *et al.* (1995) distinctive olfactory cues that snake predators associate with unpalatability, are probably enhanced ("potentiated") with bright colouration, and not vice versa when olfactory cues serve only to enhance the avoidance effect of brightly coloured prey (as it is the case in visually oriented predators, e.g. birds).

On seven occasions, six snakes (two specimens *C. laphystius*, two specimens *T. infrasignatus* and one specimen of *T. lateralis* and *C. boulengeri*) including two specimens that did not ingest any of the frogs) attacked *Mantella* frog, and released it shortly after the attack, without ingesting it. On two occasions, after releasing a *Mantella*, one snake (*C. laphystius*; the specimen that ate the five *Mantella* and five edible frogs) rubbed the mouth against the bottom of the arena, probably to remove taste substances. A similar behaviour was observed in the snake *Elaphe quadrivirgata* after attacking *Glandirana rugosa*, a distasteful frog from southern Asia (Mori 1989). In cases when *G. rugosa* was eaten by the snake,

the non-swallowing time (time without swallowing movements for longer then one second) was relatively long.

These events suggest that snakes cannot distinguish *Mantella* from edible frogs relying only on visual cues. In addition, after degustating *Mantella*, snakes did not show any interest towards *Mantella* (e.g. crossing *Mantella*, or allowing *Mantella* to sit on snake's tail and have a ride as the snake was moving; *T. infrasignatus*), but they did react to the movement of the other frog, indicating that after receiving olfactory cues, the snakes were able to distinguish *Mantella* from the other frog, probably based on the visual cues.

On several occasions, when the snake did not search actively for the prey, and did not flick with the tongue, the snake would pass near the frog without noticing it. Thus, it is considered that live prey that freeze are more likely to escape snake predation, than those that are non-moving for longer time (e.g. dead) or in relatively constant movement (Herzog and Burghardt 1974). Although snakes are considered to feed on live prey, it has been shown that in some occasions they are capable of locating non-moving prey (e.g. dead), but with higher time latency than with live moving prey (Herzog and Burghardt 1974). Despite my observations that suggest low vision ability in Madagascan snakes, diurnal colubrid snakes do possess cone retinas (Ford and Burghardt 1993), so it is likely that they have at least some capabilities for wavelength discrimination, although snake colour vision has not been experimentally demonstrated in any snake species (Burghardt 1977).

During analysis of filmed experiments, I have observed in many of the trials that the first attack happened only after the snake saw the movement of the frog, and started tongue flicking near the prey. This remark confirms the importance of prey movement for snake predation efficiency. It is well known that both vision and olfaction are important in the detection and ingestion of prey (Burghardt and Denny 1983; Drummond 1985). Visual stimuli (e.g. movement) are important in attracting snakes towards potential prey, but alone are insufficient to elicit a normal feeding response in most

species signifying that chemical information must also be received (Terrick e*t al.* 1995).

Some unpalatable frogs are not aposematic, but snake predators readily learn to avoid them based on olfactory cues (e.g. *R. rugosa*; Mori 1989). This supports the hypothesis that odour is a distinguishing trait to deter snake predators, and not conspicuousness, possibly representing an example of olfactory aposematism. On the other hand, conspicuous unpalatable prey use their aposematic colouration probably to deter bird predators, that are visually oriented. Importance of the two aposematic components (toxicity and brightness) was examined by Darst *et al.* (2006). They measured spectral reflectance of three species from genus *Epipedobates* that exhibit different levels of conspicuousness and toxicity (*E. bilinguis*- high conspicuousness, moderate toxicity; *E. parvulus*- moderate conspicuousness, high toxicity; *E. hahneli*- moderate conspicuousness, moderate toxicity). They illustrate that speed of predator learning was the greatest in the most toxic species, while conspicuousness had no effect on learning speed. On top of speed of avoidance learning is the degree to which predators avoid aposematic individuals. They demonstrated that species with moderate conspicuousness and high toxicity have the same predator avoidance as the species that are highly conspicuous, but only moderately toxic. This theory could be applied to my experienced snakes from Mantadia that learned to avoid more noxious but less conspicuous prey, and applied their experience for new, more conspicuous prey. Unfortunately, unpalatability data obtained from two bottles experiment should be considered only as a very rough approximation, for several reasons. First, I had only one skin extract from *M. aurantiaca,* so the results of the comparison based on one sample are not reliable. Second, this method was never compared to objective alkaloid data (e.g. obtained by gas chromatography). On the other hand, a greater variety and higher amounts of alkaloids in the skin do not necessarily have to mean greater noxiousness. Summers and Clough (2001) hypothesised that a contribution of toxin diversity to general noxiousness is not really known, but that the more

diverse toxin profile increases the chances of both higher lethality and palatability.

6 Predation upon *Mantella aurantiaca* in the Torotorofotsy wetlands, central-eastern Madagascar

Abstract

Malagasy poisonous frogs of genus *Mantella* are small, diurnal frogs with skin glands containing alkaloids and characterised by aposematic colouration. Due to their noxiousness and warning colouration, it is thought that they do not have many natural predators. Until now, only one successful and one aborted predation on *Mantella* frogs were reported. Herein, I account about two successful predations on *M. aurantiaca* in Torotorofotsy wetland, in central east Madagascar. The first predation was observed by lizard *Zoonosaurus* sp. and the second predation by a snake probably belonging to *Thamnosophis lateralis*. Both predators did not seem to mind the taste of the *M. aurantiaca* and ingested it.

Keywords: Amphibia: Mantellidae, poison frogs, *Thamnosophis*, *Zoonosaurus*

Only little is known about predation on poisonous frogs in general, in particular for those containing skin alkaloids. Until now, there are around 30 reports published on predation on poisonous frogs, mostly belonging to the families Bufonidae and Leptodactylidae (e.g. Guimaraes *et al.* 2004; Cuello*et al.* 2005; Menin 2005; Smith and Green 2005), and only ten published and several unpublished reports on predation and unpalatability or toxicity of frogs from the alkaloid-containing poison frogs in the family Dendrobatidae (Daly and Myers 1967; Brodie and Tumbarello 1978; Myers *et al.* 1978; Fritz *et al.* 1981; Szelistowski 1985; Hedstrom and Bolanos 1986; Master 1998; Master 1999; Summers 1999; Gray *et al.* 2002). Most of the predations observed were by snakes, and then follow predations by birds and spiders. Among the predators mentioned, there were also unsuccessful predation attempts, including the one by the large, predatory ant, *Paraponera clavata*.

Alkaloids are known to occur independently in dendrobatid frogs of New World tropics, in the bufonid genus *Melanophryniscus* of southeastern South America, in Malagasy poison frogs of the genus *Mantella* (family Mantellidae) of Madagascar, and the myobatrachid genus *Pseudophryne* of Australia (Daly *et al.* 1984; Daly *et al.* 2002). All of these frogs are also characterized by varying degrees of aposematic colouration. For Malagasy poison frogs there are only two published records of predation: Heying (2001) reported a successful predation from Nosy Mangabe in northeastern Madagascar on *Mantella laevigata* by a gerrhosaurid lizard (*Zonosaurus madagascariensis*), and an aborted predation of the same species by a boid snake (*Acrantophis madagascariensis*).

Here I report two successful predations on *Mantella aurantiaca* (figure 1), both observed in the Torotorofotsy wetlands, one of the few known sites where *M. aurantiaca* occurs. The site is located in central-eastern Madagascar, near the village of Andasibe.

The first predation event was observed by Rainer Dolch on 13 December 2004 during sunny weather. A lizard of the genus *Zonosaurus* (probably *Z. madagascariensis,* more common in this area than the superficially similar

Z. aeneus) was observed predating on and eating one individual of *M. aurantiaca* that was caught out of a group of calling males. The frog was taken away by the lizard from the site of capture to be consumed a few meters away. The reptile did not appear to be affected by any possible effects of amphibian toxins.

The second predation was observed on 22 January 2007 during sunny weather. During a field study three of us (Goran Safarek, Falitiana Rabemananjara and I) were set on the ground under a camouflage net to observe the activity and movements of the frogs. After 30 minutes, we observed a *Thamnosophis* (formerly *Bibilava*) snake. The specimen probably belongs to the species *T. lateralis* which is one of the most common species in eastern and central Madagascar (Glaw and Vences 2007). Eventually, *T. lateralis*, could be confused with *T. epistibes,* similar species which also inhabit this area. The snake started predating on and swallowing one individual of *M. aurantiaca*. The snake did not seem to mind the taste of *M. aurantiaca*, and after eating the frog, it left. This might indicate that *M. aurantiaca* toxins from Torotorofotsy are not lethal for this species. This assumption was confirmed during the snake feeding experiments performed in Andasibe in 2007 and 2008, where snakes caught in the wild were fed with two frogs at the same time (one non-conspicuous non-poisonous frog, *Guibemantis liber*, and one *Mantella aurantiaca*, caught in Torotorofotsy) giving the snake the opportunity to chose among the prey. Most of the snakes preferred non-conspicuous non-poisonous frog over *M. aurantiaca*, but those snakes that consumed *M. aurantiaca* did not show any effects of intoxication (Chapter 5).

In general, anurans are known to be preyed upon by so many predators that it has been stated that 'practically anything will eat an amphibian' (Duellman and Trueb 1994). Recently, a survey of records of vertebrate amphibian predators was published by Toledo *et al.* (2007). Based on numerous unpublished data as well as published articles and natural history notes, these authors found that snakes were the most representative group, being referred to in about 45% of the reports and should be considered the

main anuran predators. Anurans were preyed upon even when they had a large amount of skin toxins, e.g. bufonids, *Bufo proboscideus* (Menin 2005) and *Leptodactylus pentadactylus* (Roberts 1997) or highly toxic skin secretions, e.g. *Dendrobates auratus* (Hedstrom and Bolanos 1986; Master 1998; Gray *et al.* 2002), *Oophaga pumilio* (Daly, pers. comm.; Donnelly, pers. comm.), *Eupemphix nattereri* (Bezerra, 1998) and *Phyllobates terribilis* (Myers *et al.* 1978). It is also stated that birds and mammals must invest more than ectothermic predators (such as snakes) to overcome amphibian defensive strategies (Toledo *et al.* 2007). As a consequence, it is possible that snakes have been (or are) driving the diversification of anuran defensive strategies (Toledo *et al.* 2007). Probably only some of the snake and spider species are those driving the evolution of defensive mechanisms in anurans, but surely there are other groups of animals, like birds which are visually oriented predators that probably have a strong influence on the evolution of some of aposematic anuran species (Toledo *et al.* 2007).

Figure 1. Adult *Mantella aurantiaca* in its natural habitat in Torotorofotsy (S 18°52.573' E048°22.243'), Madagascar. January 2007. Photo: Goran Safarek.

7 Conclusion

Small, diurnal, aposematic frogs in the genus *Mantella* exhibit several characteristics that make them an attractive model group for studying aposematism in anurans. Contrary to dendrobatid frogs of the Neotropics, which also show aposematic coloration, *Mantella* are relatively poorly studied. Their phylogeny and alkaloid content have been studied to some extent. Knowledge about their distribution range is getting ever more comprehensive as more we study them, and some other aspects are here analyzed for the first time; tadpoles of seven species are described and their morphology compared to their phylogeny (Chapter 1), and longevity data are compared to alkaloid content of individual frogs (Chapter 2). Predation experiments on *Mantella*, both using clay models (Chapter 3) and snakes as predators (Chapter 4) were carried out for the first time. As well, the field observation on *M. aurantiaca* predation (Chapter 5) represents a rare predation report.

Tadpoles of all *Mantella* species described here show a great morphological similarity among each other and can generally be recognized easily as tadpoles of the genus *Mantella*. But due to this high similarity, a determination on a species level is difficult except for *M. laevigata* whose tadpole morphology differs from the rest of the genus. Consequently the characters of larval morphology between different species showed no correspondence with their phylogenetic relationships.

Histological bone sections of five species of *Mantella* did not show any structural differences between the bones. The longevity data obtained in this analysis confirmed a general short life span of *Mantella*, with maximum ages of two years in the wild (although it was reported that captive bred specimens can reach up to twelve years). Due to a great number of specimens that showed no distinguishable LAGs, I could not properly analyse a possible correlation between alkaloid content and age, and thus

test the hypothesis that the amount of alkaloids increases with age since *Mantella* accumulate their toxins from their diet.

Several experimental methods have been proven reliable in testing the efficiency of aposematism and clay models are one of them. Unfortunately, this method previously tested in Central and South America, showed not to be appropriate for Madagascar possibly due to a different predator composition. Despite the large number of models set in the field, I found no difference between predation and several other factors (e.g. model colour, predation time…).

On the other hand, feeding experiments in controlled conditions with snakes caught in the field showed a significant difference between predation on *Mantella* and edible, non-conspicuous frogs. From this experiment, I can conclude that bright colouration can have an aposematic function, although as a secondary trait for snake predators which probably largely rely on olfactory cues.

In general, aposematic colouration of *Mantella* frogs can be a result of several independent factors. Probably the most important factor are birds, at which this aposematic colouration may be addressed, as they are purely visually oriented predators. On the other hand, no bird predation on *Mantella* frogs has ever been observed, potentially confirming the efficiency of aposematism. A higher degree of brightness can result from sexual selection since there are unconfirmed indications that males of some *Mantella* species (e.g. *M. pulchra*) exhibit brighter colouration during the mating season. In some species, such as *M. aurantiaca*, brightness could be a sign of fitness. Namely, red colouration from *M. aurantiaca* could be related with uptake of carotenoids - the more carotenoids they ingest, the brighter colouration they have. Unfortunately, this hypothesis has not been tested yet, but could reveal another aspect of colouration in *Mantella* frogs.

Perspectives

In this dissertation I tried to give an overview of different aspects of natural history of *Mantella* frogs. I was only able to address a small part of the questions regarding this genus, and there are still many of them awaiting an answer. First of all, we need to find and describe the tadpoles of other species of *Mantella* not included here *(M. cowani, M. haraldmeieri, M. nigricans, M. manery*) and complete the descriptions of *M. madagascariensis, M. baroni* and *M. laevigata* tadpoles.

As a second big task, a comprehensive analysis consisting of brightness, unpalatability, noxiousness, longevity and population genetics should be carried out. Some new methods need to be included such as alkaloid analysis of samples collected from live specimens using an amphibian transcutaneous stimulator (TAS). This method first needs to be evaluated and compared with results obtained by only swabbing without stimulus and also with complete skin extracts. Additionally, unpalatability of skin extracts obtained in two-bottle experiments needs to be compared with objective toxicity data (LD_{50}).

Apart from these data, more information about predation on *Mantella* should be obtained. We now know how snakes react towards *Mantella*, but as I mentioned above, lizards of the genus *Zonosaurus* are also known to predate on *Mantella*. A next step would be to perform similar feeding experiments, but using *Zonosaurus* as predators, and observe their behaviour and learning abilities. In order to get more data on predation on *Mantella* in the wild, photo traps with moving *Mantella* models should be set in the field.

On the whole, I have provided additional information on the natural history of *Mantella*, such as basic morphological and longevity data and a greater insight on aposematism of *Mantella* and its deterring effect on snake predators. All the results obtained during my research provide a good starting point for future studies which can have more precise focus in order to better understand the complex system of aposematic colouration, defence mechanisms and predation on *Mantella* frogs.

8 Appendix

Table 1. List of frogs samples used in skeletochronological analysis providing location, together with GPS coordinates and voucher identification numbers, number of LAGs, SVL, sex and amoung of alkaloids (based upon total alkaloid compounds ion chromatogram intensities with 10^4 or greater = major, 10^3 –10^4 = minor, ≤10^3 = trace) .

ZCMV No	Genus	Species	Locality	Coordinates	LAGs	SVL	SEX	alkaloids-1-trace, 2-minor. 3-major
941	Mantella	aurantiaca	Torotorofotsy		1	19.5	juv	
942	Mantella	aurantiaca	Torotorofotsy		0	20.0		
943	Mantella	aurantiaca	Torotorofotsy		2	21.0	female	
944	Mantella	aurantiaca	Torotorofotsy		1	23.8	female	
945	Mantella	aurantiaca	Torotorofotsy		1	22.0	female	
946	Mantella	aurantiaca	Torotorofotsy		1	20.2	male	
947	Mantella	aurantiaca	Torotorofotsy		1	18.6	female	
948	Mantella	aurantiaca	Torotorofotsy		1	22.2	female	
949	Mantella	aurantiaca	Torotorofotsy		1	24.0	female	
950	Mantella	aurantiaca	Torotorofotsy		1	20.3	female	
951	Mantella	aurantiaca	Torotorofotsy		1	21.0	female	
952	Mantella	aurantiaca	Torotorofotsy			23.3	female	
953	Mantella	aurantiaca	Torotorofotsy		0	22.7	male	
954	Mantella	aurantiaca	Torotorofotsy			18.7	male	
955	Mantella	aurantiaca	Torotorofotsy			19.0	male	
956	Mantella	aurantiaca	Torotorofotsy			18.2	male	
957	Mantella	aurantiaca	Torotorofotsy		1	19.7	male	
958	Mantella	aurantiaca	Torotorofotsy		0	18.2	male	
961	Mantella	aurantiaca	Torotorofotsy		1	18.6	male	
1003	Mantella	aurantiaca	Torotorofotsy		0	21.8		
1004	Mantella	aurantiaca	Torotorofotsy site 2		0	20.8	male	
1005	Mantella	aurantiaca	Torotorofotsy		1	22.0	female	
1006	Mantella	aurantiaca	Torotorofotsy site 2		1	24.5		
1007	Mantella	aurantiaca	Torotorofotsy site 2		1	20.7		
1008	Mantella	aurantiaca	Torotorofotsy site 2		0	20.5		
1009	Mantella	aurantiaca	Torotorofotsy site 2		0	20.1		
1010	Mantella	aurantiaca	Torotorofotsy site 2		0	23.6		
1011	Mantella	aurantiaca	Torotorofotsy site 2		0	20.8		
1012	Mantella	aurantiaca	Torotorofotsy site 2			20.6		
127	Mantella	baroni	Ranomafana, Ranomafanakely	21° 14.921' S, 47° 22.307' E	0	25.0	male	3
128	Mantella	baroni	Ranomafana, Ranomafanakely	21° 14.921' S, 47° 22.307' E	0	20.4	sub	
129	Mantella	baroni	Ranomafana, Ranomafanakely	21° 14.921' S, 47° 22.307' E	0	24.7	male	3
130	Mantella	baroni	Ranomafana, Ranomafanakely	21° 14.921' S, 47° 22.307' E	0	23.6	male	2
131	Mantella	baroni	Ranomafana, Ranomafanakely	21° 14.921' S, 47° 22.307' E	0	23.6	male	3
132	Mantella	baroni	Ranomafana, Ranomafanakely	21° 14.921' S, 47° 22.307' E	0	21.7	male	3
133	Mantella	baroni	Ranomafana, Ranomafanakely	21° 14.921' S, 47° 22.307' E	0	27.1	female	3
134	Mantella	baroni	Ranomafana, Ranomafanakely	21° 14.921' S, 47° 22.307' E	0	24.7	male	3
135	Mantella	baroni	Ranomafana, Ranomafanakely	21° 14.921' S, 47° 22.307' E	0	22.3	male	2
136	Mantella	baroni	Ranomafana, Ranomafanakely	21° 14.921' S, 47° 22.307' E	0	22.7	male	3
137	Mantella	baroni	Ranomafana, Ranomafanakely	21° 14.921' S, 47° 22.307' E	0	24.6	male	3
138	Mantella	baroni	Ranomafana, Ranomafanakely	21° 14.921' S, 47° 22.307' E	0	23.0	male	3
139	Mantella	baroni	Ranomafana, Ranomafanakely	21° 14.921' S, 47° 22.307' E	0	23.0	male	2
170	Mantella	baroni	Ranomafana, Ranomafanakely	21° 14.921' S, 47° 22.307' E		23.5	male	3
911	Mantella	baroni	Besariaka	19° 07.718' S, 48°16.838' E	0	24.9	male	3
912	Mantella	baroni	Besariaka	19° 07.718' S, 48°16.838' E	0	27.1	male	3
913	Mantella	baroni	Besariaka	19° 07.718' S,	1	26.2	male	3

				48°16.838' E				
914	Mantella	baroni	Besariaka	19° 07.718' S, 48°16.838' E	2	26	male	3
1017	Mantella	baroni	Anosibe An'ala			25.7		
1018	Mantella	baroni	Anosibe An'ala		0	25.0		
502	Mantella	bernhardi	Manombo forest upstream		1	18.4	female	2
503	Mantella	bernhardi	Manombo forest upstream		1	18.0	female	3
504	Mantella	bernhardi	Manombo forest upstream			17.8	female	2
505	Mantella	bernhardi	Manombo forest upstream		1	15.6	male	2
506	Mantella	bernhardi	Manombo forest upstream		1	18.1	female	1
507	Mantella	bernhardi	Manombo forest upstream		1	16.0	male	2
508	Mantella	bernhardi	Manombo forest upstream		0	16.5	male	2
509	Mantella	bernhardi	Manombo forest upstream		0	16.1	male	2
510	Mantella	bernhardi	Manombo forest upstream		0	15.7	male	2
520	Mantella	bernhardi	Manombo forest upstream		0	18.3	female	3
521	Mantella	bernhardi	Manombo forest upstream		1	19.2	female	1
522	Mantella	bernhardi	Manombo forest upstream		1	18.1	female	2
523	Mantella	bernhardi	Manombo forest upstream		1	17.3	female	1
524	Mantella	bernhardi	Manombo forest upstream		1	18.0	female	2
525	Mantella	bernhardi	Manombo forest upstream		0	19.6	female	2
526	Mantella	bernhardi	Manombo forest upstream		1	18.8	female	3
527	Mantella	bernhardi	Manombo forest upstream		1	16.6	male	1
528	Mantella	bernhardi	Manombo forest upstream		1	17.8	female	2
529	Mantella	bernhardi	Manombo forest upstream		1	15.1	male	1
530	Mantella	bernhardi	Manombo forest upstream		0	16.3	male	1
531	Mantella	bernhardi	Manombo forest upstream		1	17.2	female	1
532	Mantella	bernhardi	Manombo forest upstream		0	15.6	male	2
620	Mantella	bernhardi	Manombo Camp	23° 01.699' S, 47° 43.892' E	1	18.3	female	2
621	Mantella	bernhardi	Manombo Camp	23° 01.699' S, 47° 43.892' E	1	14.3	male	2
701	Mantella	bernhardi	Vevembe		0	15.8	male	3
702	Mantella	bernhardi	Vevembe		0	15.4	male	3
703	Mantella	bernhardi	Vevembe		1	15.1	male	3
704	Mantella	bernhardi	Vevembe			15.4	male	3
705	Mantella	bernhardi	Vevembe		0	15.5	male	3
706	Mantella	bernhardi	Vevembe		0	14.4	male	3
707	Mantella	bernhardi	Vevembe		0	14.2	male	2
708	Mantella	bernhardi	Vevembe		0	15.4	male	3
709	Mantella	bernhardi	Vevembe		0	15.7	male	3
710	Mantella	bernhardi	Vevembe		0	17.4	female	1
711	Mantella	bernhardi	Vevembe		0	17.2	female	3
712	Mantella	bernhardi	Vevembe		0	16.0	male	3
713	Mantella	bernhardi	Vevembe		0	14.3	male	3
714	Mantella	bernhardi	Vevembe			15.5	male	2
715	Mantella	bernhardi	Vevembe		0	14.0	male	3
904	Mantella	bernhardi	Vevembe		0	18.6	female	3
905	Mantella	bernhardi	Vevembe		0	15.4	male	3
906	Mantella	bernhardi	Vevembe		0	15.4	male	2
907	Mantella	bernhardi	Vevembe		1	16.1	male	3
908	Mantella	bernhardi	Vevembe		0	16.3	male	3
909	Mantella	bernhardi	Vevembe		0	14.8	male	3
910	Mantella	bernhardi	Vevembe		0	15.9	male	3
1019	Mantella	crocea	Ampangadimbolana	18° 58.425' S, 48° 04.838' E	0	17.7		
171	Mantella	madagascariensis	Ranomafana,	21° 14.921' S,		22.2	female	3

			Ranomafanakely	47° 22.307' E				
172	Mantella	madagascariensis	Ranomafana, Ranomafanakely	21° 14.921' S, 47° 22.307' E		18.2	male	3
173	Mantella	madagascariensis	Ranomafana, Ranomafanakely	21° 14.921' S, 47° 22.307' E		19.4	male	1
174	Mantella	madagascariensis	Ranomafana, Ranomafanakely	21° 14.921' S, 47° 22.307' E		19.2	male	
175	Mantella	madagascariensis	Ranomafana, Ranomafanakely	21° 14.921' S, 47° 22.307' E		20.6	male	3
176	Mantella	madagascariensis	Ranomafana, Ranomafanakely	21° 14.921' S, 47° 22.307' E	0	22.4	female	3
915	Mantella	madagascariensis	Besariaka	19° 07.718' S, 48°16.838' E	1	23.6	female	3
916	Mantella	madagascariensis	Besariaka	19° 07.718' S, 48°16.838' E	0	20.9	male	3
917	Mantella	madagascariensis	Besariaka	19° 07.718' S, 48°16.838' E	1	25.0	female	3
918	Mantella	madagascariensis	Besariaka	19° 07.718' S, 48°16.838' E	1	21.8	male	1
919	Mantella	madagascariensis	Besariaka	19° 07.718' S, 48°16.838' E	2	24.9	female	1
1013	Mantella	madagascariensis	Anosibe An'ala		1	21.4		
1014	Mantella	madagascariensis	Anosibe An'ala		0	19.8		
1015	Mantella	madagascariensis	Anosibe An'ala		0	21.7		
1016	Mantella	madagascariensis	Anosibe An'ala		0	25.7		
959	Mantella	aurantiaca			1	19.1	uncertain	

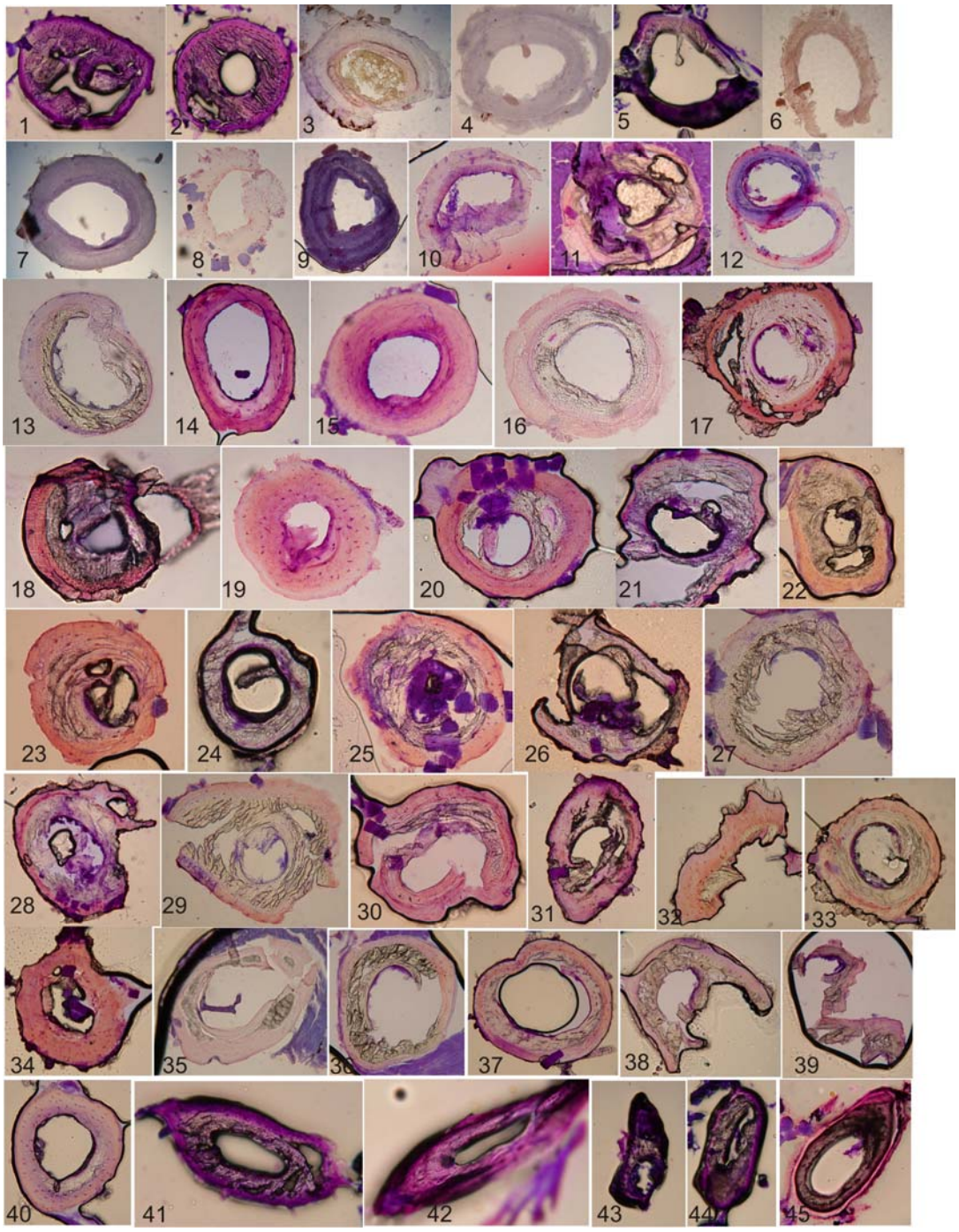

Figure 1. Histological bone sections of *Mantella* long bones obtained during skeletochronology. ZCMV voucher specimens are following: 1) 911, 2) 912, 3) 913, 4) 914, 5) 915, 6) 916, 7) 917, 8) 918, 9) 919, 10) 941, 11) 942, 12) 943, 13) 944, 14) 945, 15) 946, 16) 947, 17) 948, 18) 949, 19) 950, 20) 951, 21) 953, 22) 957, 23) 958, 24) 959, 25) 961, 26) 1003, 27) 1004, 28) 1005, 29) 1006, 30) 1007, 31) 1008, 32) 1009, 33) 1010, 34) 1013, 35) 1014, 36) 1015, 37) 1016, 38) 1017, 39) 1018, 40) 1019, 41) 502, 42) 503, 43) 505, 44) 506, 45) 507. For voucher identification, see table 1 (appendix).

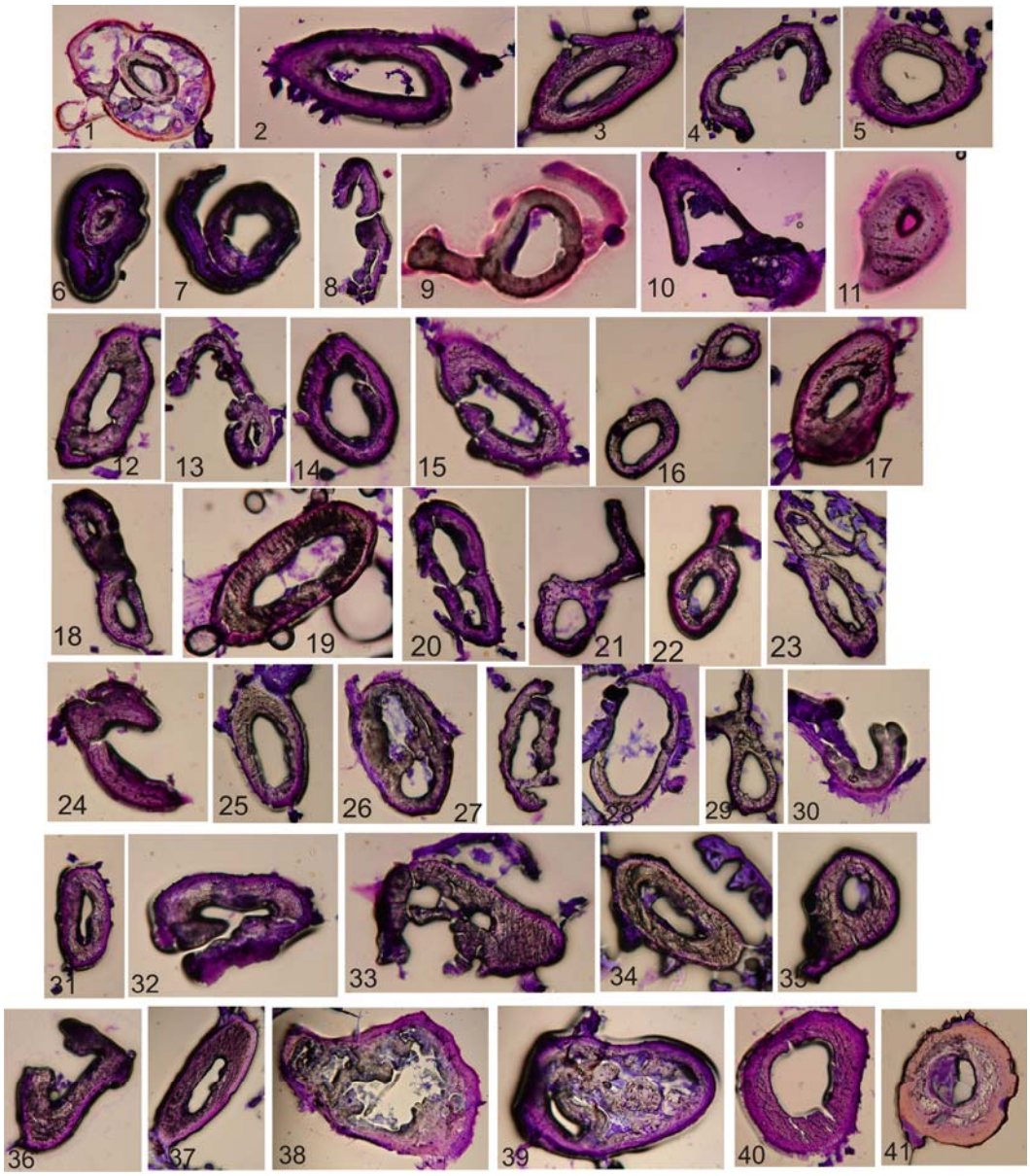

Figure 2. Histological bone sections of *Mantella* long bones obtained during skeletochronology. ZCMV voucher specimens are following: 1) 508, 2) 509, 3) 520, 4) 521, 5) 522, 6) 524, 7) 526, 8) 528, 9) 529, 10) 530, 11) 531, 12) 620, 13) 621, 14) 701, 15) 703, 16) 705, 17) 706, 18) 707, 19) 708, 20) 709, 21) 710, 22) 711, 23) 712, 24) 713, 25) 715, 26) 702, 27) 901, 28) 902, 29) 525, 30) 527, 31) 904, 32) 905, 33) 906, 34) 907, 35) 908, 36) 909, 37) 135, 38) 138, 39) 139, 40) 176, 41) 903. For voucher identification, see table 1 (appendix).

Table 2. Results for each snake separately for 2007. L. sexlin= *Liopholidophis sexlineatus*, T. latera= *Thamnosophis lateralis*, C. laphys= *Compsophis laphystius*.

Snake species	Edible/ days offered	Edible eaten	*Mantella*/ days offered	*Mantella* eaten	Average edible eaten (%)	Average *Mantella* eaten (%)
L. sexlin	10	10	15	2	1.0	0.1
L. sexlin	9	4	12	0	0.4	0
T. latera	7	7	6	5	1.0	0.8
C. laphys	11	3	21	0	0.3	0
C. laphys	16	5	14	2	0.3	0.1
C. laphys	16	5	-	-	0.3	-

Table 3. Results for each snake separately for 2008. T. infras= *Thamnosophis infrasignatus*, T. episti= *T. epistibes*, T. latera= *T. lateralis*, L. sexlin= *Liopholidophis sexlineatus*, C. laphys= *Compsophis laphystius*, C. boulen= *C. boulengeri*, I. perine= *Ithycyphus perineti.*

Snake species	Locality	Naïve yes/ no	Edible offered	Edible eaten	*Mantella* offered	*Mantella* eaten	Average edible eaten (%)	Average *Mantella* eaten (%)
T. infras	Andasibe	yes	2	0	2	0	0	0
T. infras	Mantadia	no	12	8	10	1	0.7	0.1
T. infras	Torotorofotsy	no	4	2	4	0	0.5	0
T. episti	Mantadia	no	8	4	5	0	0.5	0
T. episti	Andasibe	yes	2	2	2	1	1	0.5
T. latera	Mantadia	no	9	5	9	0	0.6	0
L. sexlin	Andasibe	yes	1	0	1	0	0	0
L. sexlin	Andasibe	yes	1	0	1	0	0	0
L. sexlin	Andasibe	yes	4	2	4	2	0.5	0.5
C. laphys	Ranomafana	yes	6	5	6	5	0.8	0.8
C. laphys	Ranomafana	yes	4	3	4	1	0.75	0.25
C. laphys	Ranomafana	yes	4	1	3	0	0.25	0
C. laphys	Ranomafana	yes	3	0	2	0	0	0
C. boulen	Ranomafana	yes	3	0	3	0	0	0
C. boulen	Ranomafana	yes	3	0	3	0	0	0
I. perine	Andasibe	yes	5	1	4	0	0.2	0

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